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Award Number: DAMD17-01-2-0038

TITLE: Novel Resuscitation from Lethal Hemorrhage-Suspended Animation for Delayed Resuscitation

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REPORT DATE: October 2003

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Final Addendum (15 Aug 2002 - 30 Sep 2003)

4. TITLE AND SUBTITLE
Novel Resuscitation from Lethal Hemorrhage-Suspended Animation for Delayed Resuscitation

5. FUNDING NUMBERS
DAMD17-01-2-0038

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: ALL DTIC reproductions will be in black and white

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

We have been working since the 1980s, for the past 5 yrs under DOD support, on novel ways to resuscitate "unresuscitable" trauma victims. We focus on combat casualties who exsanguinate internally resulting within a few min in cardiac arrest (CA). We have conceived and documented the concept of "suspended animation (SA) for delayed resuscitation" using a hypothermic saline flush into the aorta within the first 5 min of CA, using novel clinically relevant outcome models in dogs. With the use of saline flush we have achieved complete recovery after circulatory arrests of up to 120 min at 10°C. This is the report on yr 5. In yr 5, we developed strategies to make SA feasible for exsanguination CA victims. First, our initial studies on SA were promising, but the large volume of flush required presented limitations for field use. By recirculating the initial flush volume in our dog model, we were able to dramatically reduce the required flush volume from ~400 mL/kg to 50 mL/kg. Second, when severe trauma was superimposed on our SA model it resulted in coagulopathy and multiple organ failure. Using post-resuscitation plasma exchange therapy, we achieved normal outcome in some dogs after exsanguination CA of 120 min. Yr 5 included two additional lines of investigation. 1) Using a rat model of decapitation ischemia –and applying a state of the art proteomics analysis--we began to define the cascade of protein degradation that occurs despite profound cooling. This will allow us to define the limits of resuscitability, aid in the titration of hypothermia (depth, duration), and help us target the best adjunctive therapies. 2) Using a pig model of severe hemorrhagic shock, we studied emergency mild hypothermia to facilitate translation of hypothermia from the lab to clinical use in trauma settings where mild cooling rather than SA may be sufficient. We also advised industries for novel smart catheter insertion and cooling devices that we will need to bring SA to patients. We are planning clinical trials.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Clinical death. Cerebral ischemia. Coagulopathy. Traumatology	310
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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A. ABSTRACT

NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE
Suspended Animation (SA) for Delayed Resuscitation

Keywords: Resuscitation. Hypothermia. Exsanguination. Combat Casualties. Cardiac Arrest. Cerebral Ischemia. Cardiopulmonary Bypass. First Aid.

This study concerns primarily military and civilian trauma-induced exsanguination cardiac arrest (Exs CA). Most such cases are considered unresuscitable. Our suspended animation (SA) dog studies in years 1-4 (1998-2002) showed that flushing cold saline into the aorta at the start of Exs CA can lower brain temperature (Tty) very rapidly to 10°C and thereby achieve complete recovery after up to 90 min CA (no flow) (in some dogs after 120 min CA) without trauma, and after 60 min CA with trauma. Resuscitation is with cardiopulmonary bypass (CPB). Using Tty 10°C, preservation solution Unisol, and the antioxidant drug tempol, complete recovery was achieved after 120 min CA without trauma. Thirteen other drugs tested gave no breakthrough effect. In year 5 (2002-03), we propose to conduct 3 studies simultaneously, studies I and II in dogs, in preparation for clinical trials. Study I: *To maximize resuscitability* from *traumatic* Exs CA of 60 min (now only partially successful) to CA of 120 min no-flow with laparotomy, realistic trauma of the spleen, and thoracotomy for aortic cold flush to Tty 5-10°C. We will resuscitate with CPB and normalize hematocrit with whole blood. We will compare 5 groups (n 5x10) -- control group 1 with saline flush, group 2 with normosol flush, Unisol, and antioxidant tempol; and group 3 with plasma exchange after resuscitation to mitigate reoxygenation injury and coagulopathy, and prevent MOF – all with CA 60 min. Then, effective treatments will be combined to achieve intact survival after CA 90 min (group 4) and SA 120 min (group 5). Study II: *To help increase feasibility* of SA induction in the field with small groups in dogs: 1) Reducing flush volume requirement by recirculating diluted venous blood (if available) via cooler, with vs without oxygenator. 2) Exploring flush and resuscitation via thoracotomy without CPB. 3) Testing new prototype devices developed by industry (guided by us gratis): novel catheters for rapid vessel access with and without thoracotomy; a miniaturized portable cooling/pumping device for induction of mild hypothermia (34°C) under spontaneous circulation; a reservoir-pump for profound hypothermic aortic cold flush (2°C) at start of CA; and a portable CPB unit. Study III: *To start exploring the limits of resuscitability* during prolonged clinical death in rats in a basic science pilot project. To identify mitochondrial indicators, using proteomics of cell death during CA at various temperatures, without reperfusion. In year 6 (2003-04) (last year with PS as PI) we tentatively plan to document in SA dog studies the protocols for clinical trials, using lessons learned from years 1-5 – (a) for traumatic Exs CA, and (b) for normovolemic VF CA unresuscitable with CPR (now being explored in year 4). We shall document absence of MOF and intact cognitive function. We may continue the above basic science study III. This resuscitation study is coordinated with 4 additional studies (funded separately), which this team initiated and is guiding: 1) "smart catheter" (Coordinated by Dr. Yaffe and including Alion, Cedera, and CDT); 2) portable cooler (Ardiem); 3) portable CPB (Cardeon Co.); and 4) initiating clinical trials (Dr. Tisherman et al).

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PI: P. Safar

ANNUAL RESEARCH REPORT FOR USAMRMC/TATRC

NOVEL RESUSCITATION FROM LETHAL HEMORRHAGE
Suspended Animation (SA) for Delayed Resuscitation
Project Year 5

INTRODUCTION

This research report for 2002/03 concerns our US Army funded research project on novel resuscitation from severe hemorrhage, "suspended animation (SA) for delayed resuscitation" (PI: Dr. Safar. Co-PI: Dr. Tisherman), project yr 5 (academic yr 2002/03, FY-02). This report was prepared by Patrick Kochanek, MD, Director of the Safar Center for Resuscitation Research. We were all deeply saddened by the death of Dr. Peter Safar on August 3rd 2003—after a 15-mo battle with cancer. Based on the wishes of Dr. Safar and with the enthusiastic support of both the entire research team, and the industrial investigators and consultants, Dr. Kochanek assumed the role of PI of this project with Samuel Tisherman, MD remaining as Co-PI. This transition was carefully planned, over 1 yr, by Dr. Safar before his death—and included extensive discussions and involvement of Dr. Kochanek in all aspects of the project. Approval by the US Army of the plan for Dr. Kochanek to assume the role of PI on this grant is pending. Dr. Kochanek has been the Director of the Safar Center for 9 yrs and was involved with the SA project during its entire existence. Before Dr. Safar's death, Dr. Kochanek hosted a meeting of all of the investigators involved in the project—at the Safar Center on June 26, 2003. That meeting included presentations by all of the groups involved in the SA project--updating their progress. The meeting was highly successful. The experimental work carried out during yr 5 involved 4 studies in 3 separate models: Study I) SA using a small volume flush, Study II) Successful resuscitation after 2 hrs of exsanguination cardiac arrest (CA) with severe trauma, Study III)

Exploration of the limits of SA using a rat model of decapitation ischemia and state-of-the-art proteomics, and Study IV) Development of a model of lethal hemorrhagic shock (HS) in pigs and testing of mild hypothermia for its translation to clinical use. Overall, for the 4 studies, a total of 41~1 wk-long dog experiments, 10 rat proteomics experiments, and 40 24 hr pig experiments were carried out. These are described in detail below.

In this yr 5, we continued using a systematic approach, aiming for a breakthrough in resuscitation attempts for the presently considered unresuscitable condition of 2 hr traumatic exsanguination CA. In yr 1 (1998-99) we established the non-traumatic exsanguination CA model (1,2). In yr 2 (1999-00), we explored pharmacologic adjuncts to hypothermic flush, achieving no breakthrough effect with any of 14 drugs (3). Some benefit came from the antioxidant tempol (4). In yr 3 (2000-01) we pushed profound hypothermic preservation with aortic large-volume saline flush to tympanic temperature (Tty) 5-10°C; we achieved intact survival after a CA of either 60 min or 90 min at 10°C, and inconsistently after CA 120 min (5-7). In yr 4 (2001-02) we documented a 5 min limit to flush delay, pushed the limit of SA to 120 min, and documented problems with coagulopathy when severe tissue trauma was superimposed on our standard exsanguination CA protocol and SA. In yr 4, separate studies also documented the efficacy of SA in a dog model of refractory ventricular fibrillation (VF) CA—setting the stage for the use of this approach even in normovolemic CA victims with sudden cardiac death—those patients that do not respond to standard ACLS protocols.

In yr 5, using the dog model of exsanguination CA, we built upon the strong foundation of work and carried out 2 important studies. In Study I, our prior work with the SA paradigm

consistently produced survival with intact neurologic outcome after 90 min of CA, and in some dogs, after 120 min of arrest. However, large flush volumes were needed. Using recirculation of the initial flush, we were able to reduce the flush volume from ~400 mL/kg to 50 mL/kg. In Study II, we successfully tackled the critical challenge of designing an approach that allowed SA to be successfully applied to a 2-hr CA with superimposed severe trauma in dogs. We used a contemporary therapy—plasma exchange—to control coagulopathy and facilitate intact survival after 120 min of exsanguination CA with superimposed severe trauma (laparotomy, splenectomy, and thoracotomy).

In study III, we began an important additional line of investigation using a rat model of decapitation ischemia –and applying a state-of-the-art proteomics analysis. These studies were designed by Dr. Safar to begin to define the cascade of protein degradation that occurs despite profound cooling. Knowing the proteins that are injured during SA will help us define the limits of resuscitability—possibly defining a biomarker(s) for clinical use, aiding in the titration of hypothermia (depth, duration), and uncovering the best adjuncts to SA. These studies are being carried out under the direct supervision of Larry Jenkins, PhD at the Safar Center.

In study IV, using a pig model of severe HS, we carried out studies of emergency mild hypothermia to strengthen the existing data in the literature on the use of mild hypothermia in hemorrhagic shock and facilitate translation of hypothermia from the lab to clinical use in trauma settings (without CA) where mild cooling rather than SA may be sufficient. Most of the supportive data for use of mild cooling in HS were generated in rodents--additional large animal data are needed. As of the date of preparation of this report, we have studied 40 pigs—first

defining a new model of lethal HS and then studying mild hypothermia induced with either room temperature of ice-cold saline flush. Our preliminary data on hypothermia in 15 pigs are very promising. We will complete a total of 24 experiments before returning to our dog SA studies. We view this study as critical to convincing the trauma community to move forward with clinical trials of mild hypothermia in HS.

During yr 5 we had 6 publications from work on the SA project (10-16) building on a remarkable body of publications from this program (1-16). This included our important report documenting the success of SA for a 2 hr insult (14). Fellows working on this project also presented 6 abstracts of this work during yr 5 (abstracts 7-12). Drs. Kochanek and Safar also published an invited editorial in the *Journal of the American Medical Association* on a meta-analysis showing benefit of mild-moderate hypothermia in severe head injury in humans (15). In June of 2003, the International Liaison Committee on Resuscitation-including the American Heart Association—endorsed the use of mild hypothermia after VF CA in adults. This Level I recommendation was published simultaneously in the journals *Circulation* and *Resuscitation*. This development, a testament to the pioneering investigation of Dr. Safar and others, strongly supports the further development of emergency hypothermia-including both SA and mild hypothermia in HS, as proposed in our overall project. This development has re-energized the field of hypothermia in clinical care and research. Finally, on Nov 20th, 2002, we hosted the *1st annual Safar Symposium* at the University of Pittsburgh. That meeting—supported in part by this project--attracted over 120 clinicians/scientists and featured speakers on breakthroughs in resuscitation—including lectures on novel hypothermia research.

BODY OF REPORT (yr 5)

Study I) SA using a small volume flush

SA was developed to preserve the exsanguinating trauma victim for delayed resuscitation. When induced with a large volume of cold saline, SA results in intact survival after 90 min CA in dogs. However, the volumes of flush needed to induce SA (~400 mL/kg to achieve a target temperature of 10°C), greatly limit potential field application in military or even civilian trauma.

Hypothesis: A small flush volume with extracorporeal recirculation can be used to induce SA.

Methods: Dogs (n=12, 20-25 kg) were exsanguinated to CA over 5 min. At 2 min CA, SA was induced by arterial flush using Plasma-Lyte at 2°C. Four experimental groups were studied, namely 1) LT = Large volume flush administered via an 11-F balloon catheter with tip in the decending thoracic aorta (thoracic catheter); 2) LF = Large volume flush administered via a femoral cannula (large bore, 13 F); 3) ST = Small volume flush with recirculation administered via the aforementioned thoracic route; and 4) SF = Small volume flush with recirculation administered via femoral cannula. In the LT group (n=3), flush was infused into the thoracic aorta. The balloon was then deflated and the catheter withdrawn into the abdominal aorta, and the flush was continued until Tty 15°C (total flush 400 ± 100 mL/kg). In the LF group (n=3), Plasma-Lyte was infused through the cannula in the femoral artery until Tty 15°C. In the ST group (n=3), a 50 mL/kg aortic flush through the balloon catheter was followed by veno-arterial extracorporeal cooling (without oxygenator) until a Tty of 15°C was achieved--at which time the catheter was withdrawn into the abdominal aorta. In the SF group (n=3), the flush volume was identical to the ST group but was through a cannula in the femoral artery. In all groups, the flow rate was adjusted to reach the target Tty after 15 min of CA. Restoration of spontaneous circulation and assisted circulation were achieved with cardiopulmonary bypass to 2 h, after

which mild hypothermia (34°C) was maintained for 12 h, including controlled ventilation to 20 hr and intensive care to 72 hr. Outcome was evaluated using the overall performance category (OPC) 1 = normal, OPC 2 = moderate disability, OPC 3 = severe disability, OPC 4 = coma and OPC 5 = dead, and neurologic deficit score (NDS) 0-10% = normal, 100% = brain dead). In addition, we carried out perfusion fixation at 72 hr with necropsy and determination of total and regional brain histological damage score (HDS).

Results: All dogs survived to 72 h, except for one in the LT group (Table 1). In the large volume one-way flush groups (LT and LF, n=6) no dogs achieved OPC 1, whereas in the small volume recirculation groups (ST and SF) 4 of 6 dogs achieved OPC 1. OPC 3, 4 or 5 (poor outcome) was seen in 4 of 6 large volume flush dogs and 1 of 6 small volume dogs. NDS scores varied according to OPC. HDS data are pending. The difference in outcome between the small volume groups vs large volume groups was not significant (p=0.24 for OPC; p=0.08 for NDS).

Table 1. Outcomes in Study 1; SA using small volume vs large volume flush

Outcome Assessment	LT group	LF group	ST group	SF group
OPC 5 (dead)	●			
OPC 4			●	
OPC 3	● ●	●		
OPC 2		● ●	●	
OPC 1 (normal)			●	● ● ●
NDS (0%=normal, 100% =brain dead)	55,53	37,26,8	56,15,9	1,0,0

LT = Large volume flush administered via thoracic catheter; LF = Large volume flush administered via femoral catheter; ST = Small volume flush with recirculation administered via thoracic catheter; SF = Small volume flush with recirculation administered via femoral catheter.

Conclusions: SA with a small flush (50 mL/kg) volume and veno-arterial cooling to Tty 15°C enables intact survival after 90 min of CA. Surprisingly, the small volume flush outperformed large volume flush. Most likely this relates to an advantage conferred by recirculation of the flush–rather than one-way flush with a massive volume of saline. This result supports clinical feasibility of SA and obviates the need for induction with a large volume flush. Surprisingly,

small volume flush by the femoral route appears to be optimal. For civilian use of SA, this approach could be achieved in a trauma bay. In combat scenarios it could be used in portable armored far-forward vehicles or field hospitals. For military field use, we will need to develop pharmacologic adjuncts to the small volume SA flush that either enhance the protection conferred by hypothermia or facilitate the cooling effect of the small volume solution (Please see abstract 13 for a preliminary report of this study).

Study II) Successful resuscitation using SA after 2 hrs of exsanguination CA and severe trauma

We previously showed survival in dogs after exsanguination CA with superimposed severe trauma after 1 hr of no-flow using SA. However, when trauma is superimposed onto our insult, survival beyond 1 hr of no-flow is complicated by coagulopathy and multiple organ failure.

Hypothesis: The addition of plasma exchange therapy after SA with severe trauma would reverse coagulopathy and extend intact neurologic survival to 120 min of SA with no-flow.

Methods: Under anesthesia, dogs were subjected to exsanguination CA with superimposed trauma (laparotomy, splenectomy, and thoracotomy). SA was achieved with cooling to tympanic temperature of 10°C via an initial flush of 50 mL/kg saline at 4°C followed by veno-arterial cooling through a heat exchanger. Definitive surgery (splenectomy) was performed after 45 min of no-flow to simulate the clinical scenario. After a total of 2 hr of no-flow, SA was terminated and spontaneous circulation was restored using cardiopulmonary bypass at 34°C. Plasma exchange was performed in a randomized fashion thereafter (6, 20, and 40 hr). Coagulation was assessed in both groups using thromboelastography along with blood coagulation profiles.

Results: Six dogs in the plasma exchange and 7 in the no-plasma exchange group survived to 96 hr (p=NS). However, in the plasma exchange group, 3 of 6 dogs were neurologically normal (OPC 1). In the no-plasma exchange group, no dog was neurologically normal (2 dogs were OPC 2, 3 were OPC 3 and 2 were OPC 4 [severe disability], Table 2). Hypocoagulation occurred 2 hr after reperfusion (increased reaction time, decreased α-angle and amplitude $p<0.05$). Plasma exchange therapy corrected the coagulopathy (p=0.05 vs no-plasma exchange).

Conclusions: SA preserves previously lethal exsanguination CA victims for up to 2 hr when definitive surgical repair might be clinically feasible. Subsequent use of plasma exchange therapy reverses ensuant coagulopathy, enabling full neurologic recovery. Our study suggests that therapies targeting coagulopathy are needed to maximize outcome after SA in the setting of severe superimposed trauma. Further study of plasma exchange and related approaches, is warranted (see abstract 10. The plasma exchange and coagulation parameter assessments were overseen in by Drs. Joseph Carcillo, and Frank Bontempo, respectively. Blood banking for this study was carried out by Ann Hale, DVM (Stockbridge, Michigan).

Table 2. Results of Study II; Successful resuscitation after 2 hrs of exsanguination CA with severe trauma using SA with or without plasma exchange

	PEX	No-PEX
OPC 5 (Coma)		
OPC 4 (Severe disability)		● ●
OPC 3 (Moderate disability)	● ●	● ● ● ●
OPC 2 (Mild disability)	●	● ●

OPC=Overall performance category; PEX=plasma exchange; $p < 0.05$ for PEX vs No-PEX

Study III) Exploration of the limits of SA using a rat model of decapitation ischemia and proteomics

We began to examine the use of proteomics as a determinate of the time limits of brain tissue resuscitability. Two dimensional (2D) gel electrophoresis is gaining momentum as a proteomic

technique to evaluate neural injury. Complete global brain ischemia (GBI) without recirculation is an ideal model to evaluate homogeneous CNS changes since the ultrastructural responses vary little among different brain regions or cell types. We examined hippocampal proteomic changes after 30 min of GBI at normothermia vs hypothermia to identify any predictive patterns of mitochondrial protein degradation or posttranslation modification. Hypothermia at 10° C is optimal in our dog SA model—and was thus selected for use in these studies.

Hypothesis: 2D gel based proteomics provides sufficient sensitivity to assess the neuroprotective effect of hypothermia (10°C) on rat hippocampal proteins during 30 min of GBI.

Methods: After isoflurane anesthesia, complete GBI was initiated by decapitation. Hippocampi (n=6/group) were rapidly (<2 min) dissected. One hippocampus was placed into a 1 ml tube and incubated at 10°C in an ice bath for 30 min, the other hippocampus was simultaneously placed into a 1 ml tube and incubated at 38°C in a water bath for 30 min. To minimize genetic variability, we compared hippocampi from the same rat (n=5/group). Hippocampi were frozen in liquid nitrogen and stored at -70°C for 2D-gel analysis. Pooled hypothermia vs normothermia samples (all 5 rats of each group) were also compared. Isoelectric focusing with immobilized pH gradient (IPG) strips was coupled with large format (22x22 cm) slab gels for protein separation.

2D Sample Preparation: Frozen paired GBI and sham hippocampal samples were homogenized for 2 min (-20°C). Wet tissue weight was used to normalize sample loads. Buffer contained 7 M urea, 2 M thiourea, 40 M Tris, 4% w/v CHAPS, 1% w/v DTT, 2% w/v carrier ampholytes with protease inhibitors (Protease Complete 50% w/v solution), DNase and RNase. Samples were centrifuged and 25 or 50 µl of supernatant was mixed 6:1 with IPG rehydration buffer. Based on post-electrophoresis gel analysis, protein load was estimated at 30 µg/18 cm IPG gel strip (vs known protein loaded controls).

First-dimension gel: isoelectric focusing (IEF): Isoelectric focusing for the first dimension separation was performed using an IPG pHaser apparatus and 5000V 2D Power Supply. IPG strips (18 cm, pH 3–10 nonlinear) were rehydrated ("in-gel sample rehydration"). We used a ramping IPG strip-focusing algorithm (200-5,000V) for IEF (final focusing voltage of 5000V and 100,000Vh [18cm strips] or 50000Vh [11cm strips]).

Second-dimension large format gel: SDS–PAGE: The 2D Investigator apparatus was prepared for vertical SDS–PAGE using pre-cast large format Tris-tricine chemistry gels. Slab gels were electrophoresed for 5h at 20W/gel at 18°C. SDS–PAGE was performed immediately after IEF. Paired sham and GBI gels were run simultaneously, and a fifth gel with a dilute sham sample protein load co-migrated with molecular mass (M_r) and p*I* calibration markers for protein spot M_r and p*I* calculations during image analysis for each sample pair from that gel run.

Protein visualization and Mass Spectometry: 2DGel proteins were quantitatively stained using Sypro Ruby. Spot excisions were sent to a core Mass Spec facility (Univ of Pittsburgh). Proteins were selected for Mass Spec analysis, punched from the gel, digested with trypsin and subjected to MALDI-TOF mass spectrometry (see Figure 1).

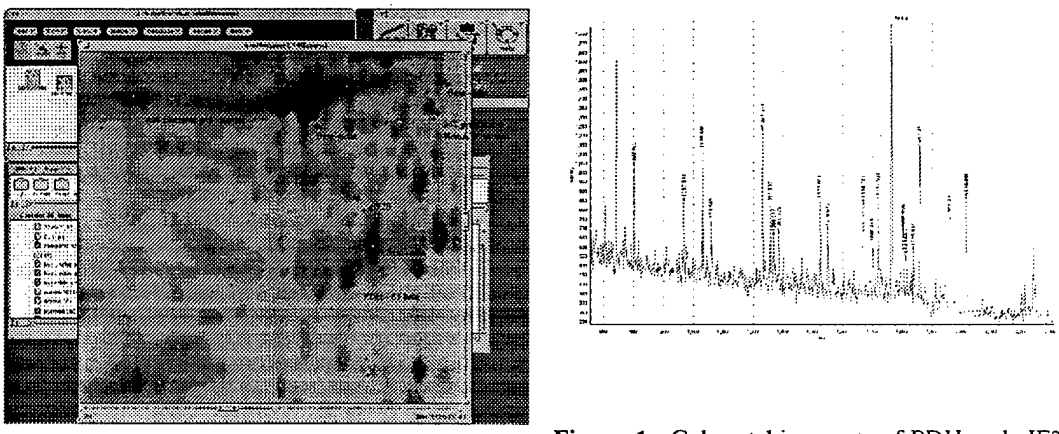

Figure 1. Gel matching spots of PDH and eIF2B in left panel with MALDI-TOF analysis of PDH spectra in right panel (see text for details).

Image analysis of 2D gels: BioImage 2D analysis software was used to locate/quantitate protein spots. Spot matching was accomplished by defining 8 common anchor spots followed by automated spot matching (constellation matching algorithm). Gel images from samples of hypothermia- and normothermia-treated GBI hippocampi were matched pair-wise to generate composite images. Composites were then matched and normalized to compensate for differences in sample loading and protein stain development. Integrated intensities of each spot on all gels were exported in tabular format for statistical analysis into a MacIntosh Statview program. Differences in intensity were calculated for each spot on every gel pair. Statistical analysis consisted of non-parametric tests. Results were reported as a list of spots that increased or decreased in intensity after GBI at a given confidence level, e.g., $p<0.01$.

Results: Spot matching with existing protein databases and selective MALDI-TOF mass spec revealed significant posttranslational and expression changes after GBI in hypothermia vs normothermia samples of proteins fundamental to the regulation of energy metabolism and protein synthesis such as pyruvate dehydrogenase (PDH) and eukaryotic initiation factor 2 beta (eIF2B), in addition to reduced cytoskeletal degradation of actin and tubulin isoforms. Furthermore, changes in oxidative defense proteins such as cooper-zinc superoxide dismutase and peroxiredoxin 2 were found (see abstracts 9 and 12). We postulate that most of these changes represent alterations in phosphorylation and oxidation state of individual proteins, during complete GBI, that are modified by hypothermia.

Conclusions: It is well known that complete GBI in the mammalian brain without recirculation produces a homogenous neuronal and glial damage, even with extended durations of ischemia. However, with recirculation, even after short durations (≥5 min) of normothermic complete GBI a heterogeneous neuronal response is seen--with classic selective neuronal vulnerability.

Mitochondrial function correlates with irreversible neuronal injury and displays a homogenous response during complete GBI but a varied response with recirculation. These ultrastructural observations suggest that a detailed examination after GBI with no recirculation would lend itself to a proteomic analysis. Our initial study sought to determine if there are specific patterns of protein changes that predict irreversible neural mitochondrial and brain tissue injury during complete normothermic GBI. Changes in key proteins related to energy metabolism, protein synthesis, and oxidative defense mechanisms were found with hippocampal gel-based proteomics during hypothermia treatment. These changes could have important roles during re-oxygenation after GBI for increasing the potential for recovery. Our preliminary data are encouraging and suggest the need for studies with longer ischemic durations and assessment of the effects of reperfusion. These proteomics studies were carried out by co-investigator Larry Jenkins, PhD at the Safar Center.

Study IV) Development of a model of lethal HS in pigs; testing of mild hypothermia

Introduction: The effect of hypothermia in the trauma and HS is still controversial. Clinical retrospective analyses suggest that hypothermia is associated with poor outcome in trauma victims, while studies in animal models of HS, mainly in rats, show that hypothermia consistently improved survival. To determine if mild hypothermia should be taken to clinical trials in humans with HS, additional studies of its efficacy are needed in clinically relevant large animal models of HS. We have used pigs to develop a clinically-relevant HS model. Our model includes 1) controlled continuous hemorrhage during shock phase, 2) no use of heparin; 3) spleen transection; 4) limited fluid resuscitation, and 5) 24 h ICU life support. The effects of mild hypothermia, induced with either room temperature or ice-cold saline, administered IV,

together with surface cooling was determined in terms of survival time, survival rate, and changes in other physiological parameters.

Method: Using anesthesia with 2-2.5% isoflurane, male pigs were intubated and ventilated. The right femoral artery was cannulated. A Swan-Ganz catheter was inserted into the inferior veno cava to measure core temperature (Tcore), and an exsanguination catheter (14-French) was inserted via the right common jugular vein into the superior veno cava. A PE 90 catheter was inserted via the left subclavian vein into the superior veno cava for central venous pressure measurement and fluid administration. A cystostomy was performed and a balloon catheter was placed. After stabilization and collection of baseline (HS 0 min), a midline laparotomy (15 cm long) was performed, which was immediately followed by blood withdrawal at 70 ml/kg/hr from HS 0-35min, 20 ml/kg/hr from HS 35-95 min, and 10 ml/kg/hr from HS 95-180 min. From HS 40 min, pigs were randomized into 3 groups: 1) normothermia group (Tcore 37.5±0.5 °C), 2) ice-cold saline hypothermia group (Tcore 34±0.5°C) and 3) room-temperature hypothermia group (Tcore 34°±0.5C). Hypothermia was induced with surface cooling and gastric lavage with ice-cold water, and infusion of test solutions. Limited fluid resuscitation for MAP >30 mmHg was initiated at HS 40 min. The test solutions were given before target temperature was achieved, then lactated Ringer's was given for control of the nadir of MAP and Tcore. At HS 3 hr, shed blood was returned, and splenectomy was performed. Life support with mechanical ventilation, iv fluid, correction of acid-base balance, and vasopressors was given for up to 24 hr. Survivors were euthanized at 24 hr.

Preliminary Results: At 24 hr, there were 2/6 survivor in the normothermia group, 3/6 in the ice-cold saline group, and 5/6 in the room-temperature group. Median survival times are 110 min in the normothermia group, 13 h in the ice-cold saline group, and 24 h in the room

temperature group. To test our hypothesis with appropriate statistical power, a sample size of 10 pigs per group is planned and studies are ongoing to achieve this goal.

Preliminary conclusion: Our data suggest that mild hypothermia increases the survival rate and survival time in a clinically relevant lethal HS model. Mild hypothermia—induced with either room temperature or ice-cold saline appears to confer benefit. Iced-saline flush in HS may more rapidly reduce Tcore; however, it may be limited by unwanted peripheral vasoconstriction.

Other accomplishments of the SA program during yr 5

Devices developments: In the mid-1990s, Safar-Klain-Stezoski and the University of Pittsburgh had received 2 patents–one on a portable heart-lung machine (CPB) with cooler (heat exchanger) and another on single or double balloon aortic catheters for emergency hemostasis, aortic flush for SA, and temperature control. These patents the University licensed to the Cardeon Co. in California. Starting in 1995, Dr. Safar presented and discussed with Safar Center associates other potentially patentable ideas: portable cooler-pump for blood cooling; iced solution reservoir for ambulances for SA by aortic flush; miniaturized tympanic thermometers; "smart" catheters (with Dr. Lyn Yaffe); transthoracic insertion; trans-thoracotomy balloon catheter; and others. Several of these devices and approaches are under development.

We established a steering committee with Dr. Lyn Yaffe as administrative chairman, to coordinate laboratory results from this Army project, developments of methods and devices, and planning clinical trials of mild hypothermia for traumatic HS and profound hypothermic aortic flush SA for exsanguination CA. This steering committee includes the Pittsburgh team (Kochanek, et al, for SA, and Tisherman, et al, for HS), Yaffe (smart catheter project), Ardiem.

(portable cooler project), and Dr. Tisherman, et al, for planning clinical trials. That steering committee met throughout the year in conference calls every week, and in person with Dr. Yaffe visiting Pittsburgh about once per month. The project of Dr. Yaffe includes Dr. Klain, and as advisors, Drs. Kochanek and. Tisherman, and Mr. S.W. William Stezoski. Weekly conference calls have proven invaluable to our team.

Several dog experiments were also used for testing of adjunctive methods and devices, before euthanasia, to save extra dog lives. These efforts (which are approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh) have led to prototypes of aortic balloon catheters that were tested in our dog experiments and are now being improved. Ardiem delivered its first prototype of a portable cooler to us in Sept. 2003. The Cardeon Co. provided regular and special aortic balloon catheters for use in dogs; but has not --due to other important priorities–been able to satisfy Dr. Safar's long-standing request to make prototypes of portable heart-lung machines with coolers.

Miscellaneous: For clinical trials, Dr. *Tisherman* continues to communicate with 6 potentially participating major trauma hospital groups. Our team carried out multiple dog and pig studies on a weekly basis. In July 2003, the large animal lab was closed for cleaning and team vacations. The weekly conference calls and lab meetings continued throughout.

Ala Nozari, M.D., Ph.D., assistant professor of anesthesiology at the Uppsala University, Sweden, started with us in summer 2001. Dr. Nozari is first author on 4 manuscripts published,

in press, or in preparation and 4 abstracts. He completed his work as a fellow and joined the anesthesia residency program at Massachusetts General Hospital, Boston.

Xianren Wu, M.D., began to serve as senior fellow on the SA project in July 2003. He has remarkable experience, particularly for a fellow, in the field of HS under the mentorship of Drs. Safar and Tisherman, including work in dog, pig, and rat models.

Mandeep Chadha, MD a fellow working under the direction of Drs. Jenkins and Kochanek is using proteomics to study protein degradation and modification during complete global cerebral ischemia with profound hypothermia. Dr. Chadha is a critical care medicine fellow who is funded separately by a T-32 entitled Pediatric Neurointensive Care and Resuscitation Research from the NICHD/NIH on which Dr. Kochanek is PI and Dr. Jenkins is a trainer. This project on proteomics in SA has broad implications across the field of resuscitation and is an outstanding opportunity for fellowship training. Dr. Chadha presented an abstract of this work at the 2003 SCCM Congress (Abstract 9). He will also present an update of this work at a fellow training session hosted by the National Center for Medical Rehabilitation Research at the NIH in Dec 2003 and at the 2003 meeting of the National Neurotrauma Society (please see abstract 12).

During yr 5, our group gave over 13 presentations on our work on the SA project. This included abstracts presented by Drs. Nozari and Chadha and invited lectures by Drs. Kochanek, Tisherman, and Jenkins.

First Annual Safar Symposium at the University of Pittsburgh School of Medicine: On Nov 20th, 2003, the *1st Annual Safar Symposium* was held at the Peterson Events Center at the University of Pittsburgh School of Medicine. This event was opened by University of Pittsburgh Chancellor Mark Nordenberg and was attended by ~120 clinicians and scientists. The morning session focused on "Breakthroughs in Resuscitation" and focused on resuscitative hypothermia. The afternoon focused on the use of simulation in resuscitation research. The symposium featured prominent national and international speakers and was supported in part by this grant. Dr. Samuel Tisherman presented a synopsis of the work on the SA project by the Safar Center at the symposium. The program is attached in the appendix.

SA project investigator and collaborator meetings at the Safar Center: In June, 2003 the Safar Center hosted a meeting of all of the participants in the SA project including industrial collaborators. The agenda included presentations by investigators from 1) the Safar Center, 2) Alion Science (with input from CDT), 3) Ardiem Medical, and 4) Cedera, Inc. Based on the success of this excellent session, we invited Drs. Calcagni and Read to the Safar Center on Oct 31, 2003 to site-visit our program. This will include presentations about both the current status and future plans for the SA project.

KEY RESEARCH ACCOMPLISHMENTS

Accomplishments for funding yr 5

Study I: We showed that small volume flush can be used in conjunction with recirculation to achieve temperatures adequate to allow SA of up to at least 2 hrs. This has important implications for potential clinical use in the field hospital, trauma bay, or armored far forward field hospital. For general field use, however, additional research is needed to try to develop

pharmacologic adjuncts that might either facilitate cooling with a small volume or augment favorable effects of cooling. These studies are proposed in yr 6.

Study 2: We showed that the coagulopathy, produced by superimposing severe tissue trauma onto 2 hrs of SA, was attenuated using serial applications of plasma exchange therapy. This therapy may improve outcomes (reduce multiple organ failure or CNS damage) in the clinical use of SA. However, we must recognize that in clinical use, unlike the situation in our dog model, many blood products are available to address coagulopathy including FFP, platelets, cryoprecipitate, activated factor VII, among other agents. Whether plasma exchange therapy is necessary after SA in clinical use remains to be determined. It deserves further study with longer durations of SA, where the putative neuroprotective benefit might be more evident. Nevertheless, it is used in some centers in the treatment of multiple organ failure and DIC and remains a potential adjunct in clinical use in the early applications of SA.

Study 3: Our initial studies applying proteomics to the SA paradigm are exciting and suggest that this technique has the potential to define key proteins that are degraded after prolonged cardiac arrest at low temperature. Our work suggests that key proteins in energy metabolism, protein synthesis, and oxidative stress are of sufficiently high copy to be assessed by this method. Future studies are needed to address the impact of reperfusion after SA.

Study 4: Our studies in a pig model of HS (without CA) strongly support the ability of mild hypothermia to prolong survival time. These studies confirm our findings in rats and will add impetus to the move to clinical trials of mild hypothermia in HS.

REPORTABLE OUTCOMES

Specific reportable outcomes for yr 5 are defined in the report and identified with an asterisk in the reference list—including publications and presentations.

CONCLUSIONS

Work in year 5 of this project has continued to build on the important studies in the first phase of our work with SA—namely—demonstration of feasibility and development of this concept. Specifically in yr 5, we have demonstrated that recirculation of the flush can dramatically reduce the flush volume required. Similarly, we tackled the challenging question of whether SA was efficacious when used in the setting of lethal exsanguination CA with superimposed severe tissue trauma. In that challenging setting, we were able to achieve good outcome in dogs when SA was combined with plasma exchange therapy—to reduce coagulopathy. We also initiated parallel mechanistic studies in a rat model of decapitation global brain ischemia to identify key aspects of protein degradation using novel proteomics methods. Finally, in yr 5, we carried out studies in pig model of severe lethal HS and our preliminary results show that mild hypothermia prolongs "the golden hour" –improving survival time. Future studies will further refine SA in our dog and rat models and mild hypothermia to optimize "Emergency Hypothermia" in lethal HS while we begin to plan and implement feasibility clinical trials.

APPENDICES

This appendix list includes all items generated by the SA project during years 1-5.
*Denotes items generated during funding yr 5. Reprints of these specific items from yr 5 are attached in the appendix.

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Lectures relevant to the SA project by Patrick Kochanek, MD

*1. Hypothermia in Brain Resuscitation: History, Present and Future. Grand Rounds, Jersey Shore Medical Center, New Jersey, January 6-7, 2003.

*2. Controversies in Neurointensive Care: Hypothermia in Brain Trauma. Society of Critical Care Medicine 32nd Annual Critical Care Medicine Congress, San Antonio, Texas, January 29-February 3, 2003.

*3. Rationale for Hypothermia for Brain Protection. Society of Critical Care Medicine 32nd Annual Critical Care Medicine Congress, San Antonio, Texas, January 29-February 3, 2003.

*4. Hypothermia in Cerebral Resuscitation. How Cold Might the Future Be? Dare to Care Congress, Cape Town, South Africa, August 24-28, 2003.

*5. Novel Potentials for Emergency Hypothermia. 2004 International Brain Hypothermia Symposium, Tokyo, Japan, February 4-7, 2004. (in preparation)

Lectures relevant to the SA project by Samuel Tisherman, MD

*1. "When is it Enough? Endpoints of Resuscitation" American College of Surgeons, New York, New York, April 12, 2003.

Lectures relevant to the SA project by Larry Jenkins, PhD

*1. "The Use of Multiple Proteomic Approaches in the Study of TBI." 1st Joint Symposium of the National and International Neurotrauma Societies (Tampa FL), October 27 - November 1, 2002 – invited speaker.

*2. Proteomics in Resuscitation Research. 1st Annual Safar Symposium, Pittsburgh, Pennsylvania, November 20, 2002.

2nd Annual Safar Symposium Program
(Please see enclosure)

SCRR Annual Report
(Please see enclosure)

Congress Review, Society of Critical Care Medicine
31st Critical Care Congress, January 26-30, San Diego, CA pp. 10-11, 2002

well as other involved parties. If the mistake was made by a trainee, joint disclosure is appropriate. In making the disclosure, he recommended expressing regret over the mistake, describing the course of the event using nontechnical language, delineating decisions that were made, and stating the nature of the mistake, the consequences, and the corrective action. "It is particularly important not to use medical jargon, to express personal regret, to apologize, and to elicit questions and concerns," he said. He added that the risk of retribution appears to be less if the patient appreciates that the physician is trying to be honest and understands that physicians are fallible. Prompt and open disclosure, an approach that attempts to diffuse anger, a sincere apology, and prompt and fair settlement as necessary also reduce potential risks to clinicians and the institution. "Disclosure is the right thing to do, but it also is necessary to help improve overall quality of care," he concluded. ■

A Family's Perspective

Most patients and families want three things in disclosure of a medical error: explanation of how it happened, evidence that the caregivers are sincerely sorry, and assurance that steps will be taken to see that it doesn't happen again, according to Nancy Conrad, Huntington Beach, Calif. Mrs. Conrad was speaking from personal experience involving the death of her husband, former astronaut Pete Conrad, after a motorcycle accident. She concurred with Dr. Wu's assessment that information should come directly and in person from the individual most responsible for the patient's care. She also advocated creating an institutional culture in which reporting medical errors is encouraged. "Many adverse events can be prevented by recognizing awareness, accountability, ability, and action," she said. "Creating the ability to intervene and aligning the incentives and conditions to prompt positive action are important." She concluded by noting that the solution to reducing medical errors may lie in the development of better systems that will minimize errors and harm and in creating a partnership for care between patient and clinician. ■

Ethical Perspectives in Disclosing Errors


Michael Williams, MD

"Empiric research shows that compliance-based programs alone are not effective in promoting behavioral change or cultural commitment," stated Michael Williams, MD, Johns Hopkins Hospital, Baltimore, Md. "This external, imposed, legalistic, regulatory approach is viewed by everyone working within the system with cynicism."

At the core of the medical error and disclosure process is respect for colleagues, patients, and families. A nonpunitive approach to medical errors means that the institution will not punish those who are participants in errors and report them in good faith, although Dr. Williams admitted that there are limits to the nonpunitive approach created by external laws. He added that this also means that health professionals and employees should not punish or blame one another if an error occurs or is reported in good faith. "The purpose of the nonpunitive approach that encourages reporting of errors is to identify a pattern and keep the near-misses and harmless hits from becoming errors," he said.

In adding to the previous speakers' suggestions for disclosing errors to patients, he advocated telling the truth, but telling it wisely. The first priority is to care for the patient, but this should be followed by obtaining the facts, determining whether there was a departure from the standard of care, and ascertaining whether the patient was harmed. He warned particularly against disclosing speculation as if it were the truth. "If there is uncertainty as to the cause of events, disclose that," he said. He concluded, "The measure of our success is not necessarily whether errors occur, but how we choose to respond to them when they occur." ■

From the ABCs to Proteomics: Hunting for the Next Breakthrough in Brain Resuscitation

Although hypothermia has been shown in the laboratory to preserve neurons following brain injury, these benefits have not translated into clinical success. Patrick M. Kochanek, MD, FCCM, Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, Pa, suggested that the problem may lie in an inability to determine all of the actions of hypothermia. "To produce a breakthrough, we really need to know how the most effective experimental therapies are working," he said. One potential solution is proteomics, a two-dimensional gel technique that separates proteins by both mass and isoelectric

pH, allowing simultaneous assessment of the effect of injury or therapy on more than 2,000 proteins. Using this technique, researchers can identify a specific number of proteins in a gel of, for example, mouse brain tissue, employ matching software that compares it with an established proteome for that tissue, identify and quantify areas of lower protein abundance, and use other methods to define specific posttranslational modifications of proteins.

Delivering the Asmund S. Laerdal Memorial Lecture, Dr. Kochanek described preliminary findings in immature rats from the first application of proteomics in experimental traumatic brain injury by Dr. Larry Jenkins at the Safar Center, using an injury level that produced little loss of hippocampus neurons. Proteomics documented considerable reduction in cytoskeletal proteins and a marked increase in the number of endogenous neuroprotectants, such as copper/zinc superoxide dismutase. (Dr. Jenkin's seminal work is now in press in the *Journal of Neurotrauma*.) There also were substantial alterations in cell-signaling proteins, particularly those affecting calcium homeostasis. A gel stained with an antibody directed against the phosphorylation motif of protein kinase B showed a loss of phosphorylation following injury that was consistent with loss of this neuroprotective cascade. In adult rat brain models, which are closer to the standard model of brain injury, proteomics documented obvious losses of proteins, especially high-molecular weight alkaline proteins, many of which are cell-signaling proteins. Further, the degree of protein degradation was greater than in the immature rat brain. "Our hope is that this technique will aid in unraveling some of the key mechanisms of secondary damage in brain injury, possibly documenting timing of degradation and interaction of various pathways," he concluded.

Another exciting area of research in traumatic brain injury is the use of "suspended animation" in the presence of uncontrolled hemorrhagic shock and rapid exsanguination. In experiments conducted by Drs. Peter Safar, Samuel Tisherman, and colleagues at the Safar Center, after 5 minutes of rapid exsanguination, animals received a shock to ensure cardiac arrest of varying durations, followed in 2 minutes by an aortic flush of solutions of different temperatures to lower brain temperature. At the end of the arrest interval, animals were resuscitated with cardiopulmonary bypass and received 72 hours of intensive care. In one set of animals, room temperature saline flush resulted in a brain temperature of 36°C, and 4°C saline flush resulted in a brain temperature of 34°C. Following a 20-minute cardiac arrest, a number of animals that had the lower brain cooling completed a normal recovery. In another experiment of 60-minute arrest time, the volume of flush was regulated by the degree of cooling desired (eg, 10, 15, or 20°C), with a volume of almost 500 mL/kg required to reach the lower temperature. One of the problems associated with using a flush for targeted brain cooling in extremely long arrest durations was adverse effects on other organs that were not cooled. This was addressed by flushing the brain to reach the desired temperature, then pulling back the catheter during the remainder of the treatment period to flush to other vital organs. Dr. Kochanek reported the following conclusions from these preliminary experiments: 1) the flush cannot be delayed more than 5 to 8 minutes following cardiac arrest, 2) flush temperatures and volumes are important regulatory factors, and 3) balloon position becomes important at long arrest times. "These factors determine the onset, depth, and distribution of hypothermia," he explained. He concluded, "Ice-cold large-volume aortic flush is ready for clinical trials in those patients with hemorrhagic shock-induced cardiac arrest in whom there are no other alternatives." ■

Ice-cold large-volume aortic flush is ready for clinical trials in those patients with hemorrhagic shock-induced cardiac arrest in whom there are no other alternatives.

Medical Management of a Chemical Terrorism Event

In the wake of the September 11 terrorist attack and subsequent anthrax mailings, United States citizens have a heightened awareness of their potential vulnerability. However, the health system is not unprepared for future attacks, and preparedness in individual institutions need not be expensive. Addressing the issues and developing a plan can prevent a chemical terrorism attack from becoming a disaster.

An Overview of Chemical Weapons

Chemical agents are divided into toxic agents, which produce injury and death, and incapacitating agents, which have temporary effects. The toxic agents are comprised of nerve agents (anticholinesterases), lung-damaging agents (choking agents), blistering agents, and blood agents (cyanogens). According to

International Congress Series 1242 (2002) 215–227

Development of cardiopulmonary–cerebral resuscitation in the twentieth century

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Abstract

Before the renaissance, death was to be *accepted* as an act of God. From then on, there was a *will* to attempt resuscitation. The *ability* to reverse coma-induced airway obstruction, apnea, and pulselessness began in response to accidents caused by general anesthesia in the late 1800s. Around 1900, knowledge existed about the majority of CPR steps. This knowledge, however, was then not assembled into an effective system because of lack of communication between laboratory researchers, clinicians, and rescuers. Open-chest CPR was effectively practiced in operating rooms during the first half of the 20th century. Anglo-American anesthesiologists co-pioneered trauma resuscitation during World War II. Modern cardiopulmonary–cerebral resuscitation (CPCR), which is now giving every person the ability to challenge death anywhere, has been developed since the 1950s. Through research in Baltimore, the chest-pressure and back-pressure arm-lift methods of artificial ventilation, taught for 100 years, were replaced by backward tilt of the head and direct mouth-to-mouth ventilation, and emergency artificial circulation by sternal compressions was rediscovered. Steps A–B–C of basic life-support were extended—to advanced and prolonged life-support. Anesthesiologists pioneered hospital ICUs almost simultaneously on three continents. In the 1960s and 1970s, several groups initiated CPR education research, the development of training aids, [illegible] resuscitation delivery through emergency medical services (EMS) systems, and the multidisciplinary specialty of critical care medicine (CCM). Since the 1970s and 1980s, cerebral resuscitation potentials after prolonged cardiac arrest have been evaluated with ICU outcome models in large animals and in randomized clinical outcome studies. Pharmacologic strategies have given relatively disappointing results. Mechanism-oriented research escalated. Postarrest CBF promotion improved outcome in animals and patients. A breakthrough came in the 1980s and 1990s with the revival of research into therapeutic hypothermia. Mild resuscitative postarrest hypothermia (which is simple and safe) showed a breakthrough effect, extending the normothermic arrest reversibility limit from 5 to 10 min no-flow. Clinical trials of mild hypothermia are being reported now, with positive results. Animal research has begun into "suspended animation for delayed resuscitation" for

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PII: S0531-5131(02)00775-6

temporarily unresuscitable cardiac arrest. Education research, delivery programs, and case registries for ongoing outcome evaluation should get higher priority.

Keywords: Cardiac arrest; Critical care medicine; Hypothermia; Resuscitation; Suspended animation

1. Introduction

The importance of attempting resuscitation is obvious from the fact that about one-quarter of all people who die on earth do so acutely, unexpectedly, before their time has come. In about one-half of these cases, resuscitation attempts are justified, whether inside or outside of the hospital. Effective emergency cardiopulmonary–cerebral resuscitation (CPCR) and intensive (critical) care medicine (CCM), and emergency medical services (EMS) for their delivery did not exist outside operating rooms before the 1950s [1–3]. Starting with definitions, we must differentiate between *protection*, which is pre-treatment; *preservation*, which is intra-insult treatment; and *resuscitation*, which is to reverse the insult.

Before the 1500s, death was *accepted* as a will and act of God. With the Renaissance, however, there came a *willingness* to resuscitate. Only around 1900 came the *ability* to resuscitate, although much of that opportunity was missed. Andreas Vesalius of Padua became the father of modern resuscitation [4]. He appreciated the importance of his discoveries, including intra-tracheal ventilation to reverse asphyxiation to near-cardiac arrest, and even ventricular fibrillation (VF), but his conservative colleagues declared him mad. To avoid execution by the Inquisition, he made a pilgrimage and died in a shipwreck.

The ability to resuscitate came around 1900, through research provoked by accidents of general anesthesia. For respiratory resuscitation outside hospitals, however, around 1850, Marshall Hall created a setback of 100 years when he told the public that positive-pressure ventilation by mouth-to-mouth or bellows can cause lung rupture. For artificial ventilation, he and his followers instead recommended chest-pressure arm-lift methods, such as the Silvester supine chest-pressure arm-lift method, Howard's supine chest-pressure only method, Schaefer's prone chest-pressure only method (taught by the American Red Cross throughout two world wars), and the Holger Nielsen method which added arm-lift to prone chest-pressure.

For respiratory resuscitation inside hospitals, although upper airway soft tissue obstruction was not fully understood, surgical textbooks of the 1870s recommended forward displacement of the mandible [5] or pulling the tongue forward by forceps and ventilating with chest-pressure arm-lift. Positive pressure ventilation of anesthetized patients did not become commonplace until after World War I, when anesthesia machines were equipped with bellows or bags. McIntosh [6] introduced an air draw-over ether vaporizer with bellows. This was modified with the self-refilling Ruben bag–valve–mask unit, which led to the portable military tri-service anesthesia and resuscitation field apparatus [7]. Tracheotomy and tracheal intubation deserve a separate history talk [8].

For *cardiac resuscitation* in chloroform arrested animals, German surgeons explored artificial circulation with or without thoracotomy, starting in the 1880s. Schiff [9] blew into the trachea, opened the chest, compressed the animal's heart by hand, and recorded a pulsewave. In 1900, Igelsrud of Norway performed the first successful open-chest resuscitation in a patient, who arrested under chloroform. In 1878, Boehm of Germany had published the ability to circulate blood in cardiac arrest of animals without opening the chest. He revived cats from potassium-induced cardiac arrest, using bellows for ventilation and chest compressions for heart massage [10]. In 1892, Maass [11], of Koenig's department in Goettingen, was the first to document a closed-chest CPR method in patients. Maass used Brosch's modification of the Silvester method [12], i.e., chest-pressure arm-lift: "I exerted expiratory chest-pressure more forcefully to the region of the heart . . . after one hour of chest compressions the radial pulse returned . . . after 10 days of stupor the boy was well." This breakthrough remained dormant for half a century.

Around 1900, Prevost and Batelli [13] of Geneva were the first to show in animals that low electric currents through the heart, externally or internally, can throw the heart into VF, while high currents can stop VF [13]. The importance of this was not appreciated until 40 years later. Crile [14] of Cleveland was the first to show that adrenaline can help restart heartbeat during heart massage.

In Pittsburgh in 1961, we assembled an effective CPCR system [15] because it was obvious that the reversal of airway obstruction, apnea, and cardiac arrest requires a combination of steps A–B–C, to be followed up with steps D–E–F of advanced life-support for restoration of spontaneous circulation (ROSC), and steps G–H–I of prolonged life-support (Fig. 1). Outcome is determined by the weakest step. A survey of this system shows that by around 1900, the only elements missing were manual airway control by backward tilt of the head and intensive care life-support. All of this knowledge that existed around 1900 remained fruitless for half a century, I believe for the following five reasons [1–3]: (1) the inventors failed to appreciate the importance of what they discovered; (2) there was no communication among clinicians, laboratory researchers, and field rescuers; (3) the system was not assembled and incomplete; (4) powerful professors resisted change; and (5) documentation with physiologic measurements was then not possible.

From around 1900 to 1960, open-chest CPR was practiced frequently, and with excellent results, in operating rooms [16,17]. In 1947, Beck et al. [18] of Cleveland performed the first successful human defibrillation, with AC, by open-chest technique. Beck also coined the term "hearts too good to die," to which in 1970, Safar added "brains too good to die." University of Pennsylvania Anesthesiology Chairman Dripps, taught a half century ago how to perform open-chest CPR, not only inside but also outside the operating room—but not outside the hospital [17]. Gurvitch and Yuniev [19] of Moscow had pioneered closed-chest countershocks with direct current in dogs in the 1940s. In the 1950s, Zoll et al. [20] of Boston used alternating current for the first time in patients for external defibrillation. Kouwenhoven used AC and DC in dog experiments. Lown of Boston introduced external defibrillation with DC into the USA, and introduced cardioversion.

In October 1956, James Elam, then an anesthesiologist in Buffalo, met Safar, then an anesthesiologist at Baltimore City Hospital. Elam et al.'s [21] measurements on anesthetized patients with mouth-to-mask or tube ventilation, along with his interest in first aid, ignited Safar's interest in resuscitation research, which he has pursued ever since. Elam et

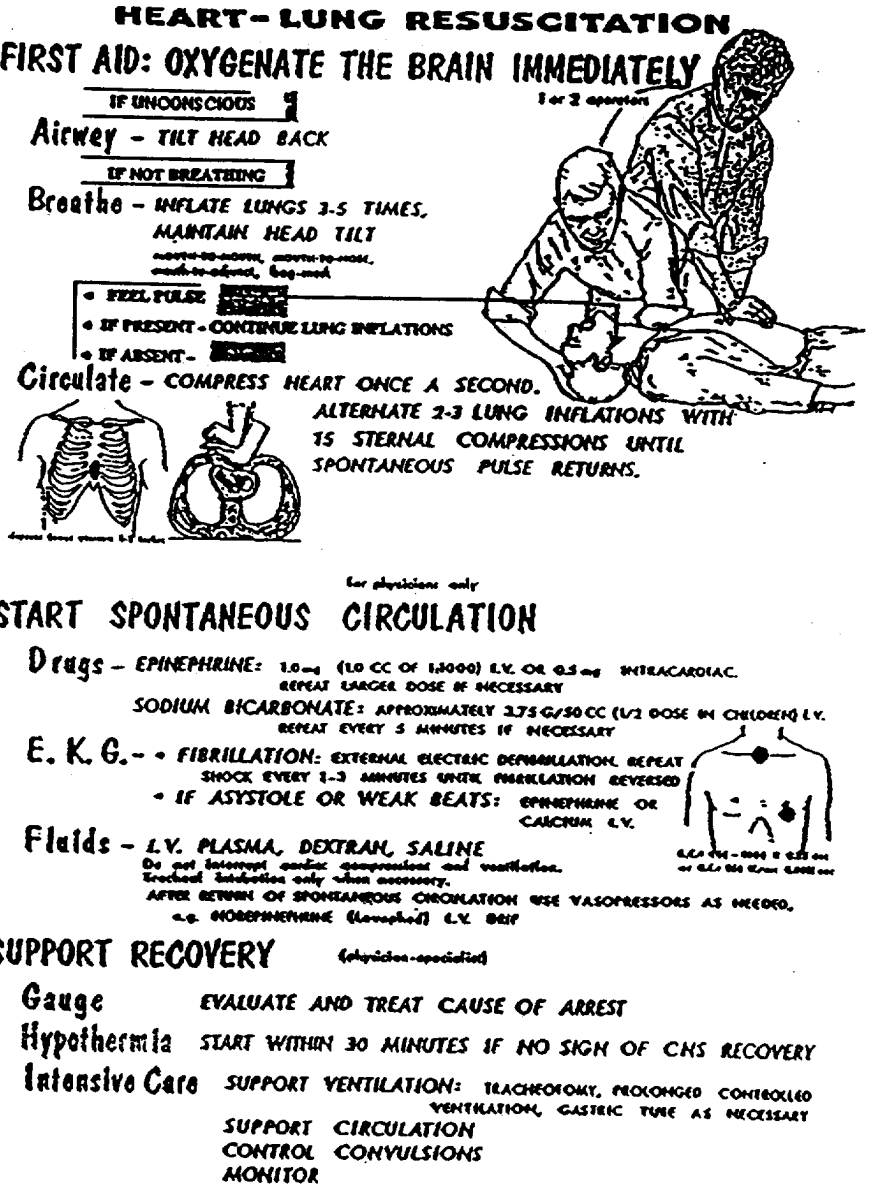

Figure 1. Heart-lung resuscitation (cardiopulmonary-cerebral resuscitation). First composition in 1961, Pittsburgh, PA. Reproduced with permission from Safar P: Community-wide CPR. *J Iowa Medical Society* 1964 (Nov); pp 629–635.

Fig. 1. The first assembly of the cardiopulmonary–cerebral resuscitation (CPCR) system in 1960 [15], with three phases of three steps each. "H" was for hypothermia and "humanized (i.e., brain-oriented) intensive care" [40].

al.'s [21] data, obtained on anesthetized patients, showed that exhaled air is adequate resuscitative gas, provided inflations are delivered with twice-normal tidal volumes. Since no one had taken Elam et al.'s data further, in December 1956, Safar embarked on comparisons of mouth-to-mouth with manual ventilation methods, without tubes or masks, in sedated curarized apneic adults. Safar considered the data of 1950 on curarized human volunteers by Gordon as invalid because they were obtained with tracheal tubes [22]. In Safar et al.'s [23] and Safar's [24] experiments, lay persons performed mouth-to-mouth and trained rescuers performed the manual methods. More than 30 physicians and medical students volunteered to be made apneic for several hours without tracheal tube. At that time, there were no human experimentation committees, but department chairmen were asked to approve any studies, and volunteers were highly informed. Safar et al.'s and Elam et al.'s resuscitation studies in the 1950s and 1960s were supported by the US Army.

On step A, in patients and volunteers, upper airway soft tissue obstruction in coma was 100% unless the head was tilted backward, to lift the tongue and epiglottis off the air passage. In addition, some victims required jaw thrust and open mouth, the triple airway maneuver [23–25]. On step B, all methods of manual artificial ventilation were associated with various airway obstruction patterns [23,24,26]. The Brosch modification of the Silvester method was relatively more effective as it kept the head tilted backward by elevating the shoulders [26]. The oldest method, mouth-to-mouth, was the winner [21–28]. Untrained lay persons delivered large tidal volumes in all cases and, after apnea to let arterial oxygen saturation decrease, rapid reoxygenation was possible with five deep inflations [24]. Gordon et al. [28] confirmed the superiority of mouth-to-mouth over manual methods in children. Within 1 year, Elam et al. [21,27], Safar et al. [23–26] and Gordon et al. [28] persuaded the world to adopt the "tilt and blow" technique in teaching first aid. Also in the late 1950s, Ruben of Denmark introduced the self-refilling bag–valve–mask unit.

In 1957, William Kouwenhoven, then professor emeritus of electrical engineering at the Johns Hopkins University, who had been engaged for many years in experiments in dogs on VF and immediate defibrillation, visited Safar during one of his steps A–B experiments. Both discussed how one might produce *artificial circulation* without opening the chest. Neither one knew about Maass. Safar asked his associate Redding to try, in dogs, to move blood with high-pressure ventilation, but this method was abandoned when it resulted only in trickle flow. Redding later pioneered our understanding of the mechanism by which epinephrine is effective in cardiac resuscitation. Knickerbocker was then a PhD research fellow with Kouwenhoven, and Jude was a surgical resident with Chairman Blalock. In 1958, Knickerbocker rediscovered external cardiac massage with a serendipitous brilliant observation on a dog: during an experiment of electrically induced VF and immediate external defibrillation, he observed an arterial pulse wave when he pressed the defibrillation paddles on the chest. Kouwenhoven et al. [29] immediately recognized the importance of this observation and documented the technique in dogs, and Jude et al. [30] in patients, with the help of anesthesiology Chairman Benson, and cases of accidental pulselessness from the newly introduced halothane. Safar et al. [31] demonstrated in 1960 that with or without cardiac arrest, with or without tracheal tube, sternal compressions *alone* can ventilate animals, which have straight airways—but not patients, who have kinked airways. They therefore combined step C with the previously established steps A and B, into phase I, basic life-support [15,31].

The rediscovery of artificial circulation by chest compressions is reminiscent of Fleming's rediscovery of penicillin. Neither Billroth of Vienna and Florey of France, who had described a penicillin effect 70 years earlier, nor Maass who resuscitated humans with external cardiac resuscitation 70 years earlier, appreciated the importance of their observations.

Intensive care for prolonged life-support became reality during a poliomyelitis epidemic in Copenhagen in the early 1950s, when anesthesiologist Ibsen [32] changed artificial ventilation from use of Drinker's iron lung, which was clumsy and often ineffective, to intra-tracheal intermittent positive pressure ventilation. Similarly, Nilsson [33] pioneered the modern treatment of drug overdose with prolonged intra-tracheal ventilation.

In 1958, Safar initiated at the Baltimore City Hospital the first medical–surgical ICU which was staffed around the clock by anesthesiologists, surgeons, and internists; and which was for *any* life-threatening vital organ systems failure [34]. He made the first major switch in the USA from the iron lung to modern mechanical positive-pressure ventilation [35]. Almost simultaneously with the ICU in Baltimore, Holmdahl [36] of Uppsala, followed by other European anesthesiologists, Spence of New Zealand, Fairley of Canada, and several other anesthesiologists in the USA, created various kinds of ICU programs. In 1968, internist Weil, anesthesiologist Safar, and surgeon Shoemaker initiated the Society of Critical Care Medicine. The first multidisciplinary physician fellowship training program in critical care medicine, initiated in Pittsburgh by Safar in 1962 and led by Grenvik [37] since 1971, has graduated so far more than 500 physician fellows from all over the world. Beecher in Boston and Grenvik in Pittsburgh pioneered brain death certification.

Modern intensive care life-support would be unthinkable without the pioneering work of Severinghaus and Bradley [38] who not only created the first tri-electrode unit for blood gas and acid base analyses, but also created clinically useful nomograms and produced much of the knowledge behind the pulse oximeter, a recognized breakthrough device. Moreover, Severinghaus was and still is a world renowned physiologist, and he remains a mentor for numerous leaders in academic anesthesiology.

The first CPR guidelines were developed in the early 1960s by the American Heart Association CPR committee-initiated by Jude, Elam, Gordon, and Safar [39]. The first international guidelines [40] on cardiopulmonary–cerebral resuscitation (CPCR), developed for the World Federation of Societies of Anaesthesiologists (WFSA) and sponsored by Laerdal, were also initiated in the 1960s. They were usually 10 years ahead of AHA guidelines and included traumatologic resuscitation and cerebral resuscitation.

Although CPR is a breakthrough discovery, it has so far delivered suboptimal results. Life-supporting first aid (LSFA), which includes steps A–B–C plus basic trauma care, was initiated as a concept in the 1960s by Laerdal and Safar [40,41]. LSFA by bystanders is still the weakest link [41]. The goal is LSFA skill acquisition by every fit human being on earth, as part of general education in hygiene. This goal, recommended by this author since the 1960s, is still elusive. Automatic defibrillation, pioneered by Mirowsky et al. [42] for implantable defibrillators, has become external as a promising addition, even in the hands of lay persons, to achieve earliest possible ROSC, as recommended since 1960 [29–31].

Emergency medical services (EMS) for CPCR delivery encompasses the life-support chain—from the scene via transport to the most appropriate hospital [43]. Its history deserves a separate talk. Briefly, Europeans were ahead of Americans with mobile ICU ambulances. The first ambulance services delivering ALS were physician-staffed mobile ICUs in Prague, Moscow, Magdeburg and Western Europe. In the USA, Safar, with Baltimore ambulance leader McMahon, created a mobile ICU vehicle in 1958. In the 1960s, Nagel et al. [44] of Miami pioneered physician control of paramedics via radio and Pantridge of Belfast using a physician-staffed mobile cardiac ICU moved electrocardiography and defibrillation into the field. Safar (followed by Nagel et al.) initiated and chaired the first ASA committee on acute medicine which authored the first guidelines for community-wide EMS organization for the delivery of a life-support chain [43]. Simultaneously, a life-support chain was promoted by Ahnefeld in Europe.

Modern EMS systems were developed since the 1960s in industrialized countries in response to several suspected triggers. Among them, Nagel believes that the chance rediscovery of step C of CPR was most important. Safar credits Anglo-American traumatologists of World War II, research in the 1950s which led to the CPR system, and the mobile ICUs in Europe. Paramedics-staffed ambulances in the US [44,45] (e.g., Seattle under Cobb and Eisenberg with so far best results for cardiac arrest cases) reach the scene faster than physician-staffed ambulances in Europe. The latter have had so far less impact on sudden cardiac death, but seem to be more effective for cases of severe polytrauma. The needed integration between prehospital and in-hospital life-support was pioneered in Europe mostly by anesthesiologists, and in the USA by the new specialty of emergency medicine. The difference seems to be caused primarily by economic factors. Anesthesiologists' contributions to traumatologic EMS were extended by Frey of Mainz, who in 1976 initiated the Club of Mainz for Emergency and Disaster Medicine [46]. Safar's group initiated "disaster reanimatology research." Recently, the International Trauma Anesthesia and Critical Care Society (ITACCS) has begun effectively to bring anesthesiologists back into traumatology.

Modern CPCR methods were first developed in the USA, but simultaneously, Negovsky [47] of Moscow has studied lifelong, since 1937, the pathophysiology of dying and reanimation. With his method of arterial infusion of oxygenated blood with epinephrine, Negovsky's team successfully resuscitated some clinically dead soldiers in the outskirts of Moscow during the German siege in World War II. Negovsky et al.'s [48] documentation of the postresuscitation disease stimulated many researchers, including Safar's group, which led to cross-fertilization between Pittsburgh and Moscow throughout the cold war.

Cerebral resuscitation for cardiac arrest has a brief history which, however, had a spark already around 1910 when Guthrie, past physiologist of Pittsburgh, considered the brain as the target organ of resuscitation [1–3]. Around 1970, Hossmann and Kleihues [49] of Cologne reported that in animals, many cerebral neurons can tolerate up to 1 h of normothermic no-flow. Unfortunately, not all neurons, because the postischemic-anoxic encephalopathy damages selectively vulnerable neurons in selectively vulnerable regions. In the early 1970s, Safar, encouraged by Hossmann's findings, coached Lind, Snyder, and Nemoto to document in monkeys and dogs the post-cardiac arrest sequence of transient cerebral hyperemia followed by delayed, prolonged inhomogeneous hypoperfusion, which is mismatched to increasing oxygen demand [50,51]. In 1974, a special treatment after

prolonged cardiac arrest and restoration of spontaneous circulation was found for the first time to improve conscious survival in dogs, i.e., postarrest cerebral blood flow promotion [52]. The post-ROSC hypertensive bout is clearly associated with better cerebral outcome, based on mechanism studies by a dozen investigators, dog outcome studies in Pittsburgh [53], and four retrospective clinical correlation studies [54]. The heart–lung machine became a useful experimental tool to control blood pressure, flow, composition, and temperature—even after very prolonged no-flow states. Use of unique large-animal outcome models led to clinically relevant new knowledge. Long-term life-support proved necessary, recognizing that the brain's recovery from an ischemic or traumatic insult is greatly influenced by extracerebral organ function, while the encephalopathy "matures" over 3 days and longer. Numerous neuroscientists have joined these efforts since the 1980s, in attempts to clarify the mechanisms of the very complex postischemic encephalopathy [55,56].

Explorations of pharmacologic strategies for cerebral resuscitation since the 1970s have yielded no breakthrough effects [55,56]. In 1979, Safar initiated the first multicenter international randomized clinical outcome study of sudden cardiac death and CPCR, the NIH-supported Brain Resuscitation Clinical Trial of 1979–1994 (BRCT) [57–59]. Postarrest barbiturate loading, calcium entry blocker therapy, or escalating high doses of epinephrine gave suggestive cerebral outcome benefit only in subgroups. We learned about the limits of randomized clinical outcome studies and found that they are not the gold standard for documenting novel treatment effects for cerebral resuscitation.

The breakthrough for the brain came from a revival of *resuscitative hypothermia*. We now know that its beneficial mechanism is not merely the reduction in oxygen demand, as documented in the 1950s, by Bigelow et al. [60] of Toronto and Rosomoff [61] of New York and Pittsburgh, but rather synergism of suppression of many deleterious cascades [55]. In 1950, Bigelow introduced moderate hypothermia (30 °C) for cerebral protection–preservation during heart surgery; Rosomoff for brain surgery, and White and Albin [62] for spinal cord trauma. The classic text on the physiology of hypothermia edited by Dripps and Severinghaus in 1956 [63] includes knowledge recently re-discovered. Around 1960, Benson et al. [63] explored it in patients. In spite of promising case reports [63,64], moderate hypothermia after cardiac arrest was then abandoned for 25 years, probably because of uncertain benefit and because it can cause arrhythmias, coagulopathy and infection, and is difficult to induce. In the early 1980s, Safar's group in Pittsburgh, disappointed with drug trials against the encephalopathy after cardiac arrest, re-initiated research into resuscitative hypothermia with controlled outcome studies [65]. In 1987, Safar made the chance discovery that after 7–15 min VF no-flow in dogs, complete cerebral recovery correlated with accidental mild hypothermia (34–36 °C) present at the start of arrest [66]. This observation was followed by five large-scale dog outcome studies with mild resuscitative hypothermia, all of which documented highly significant outcome benefit [67,68]. Simultaneously and independently, neuroscientists in Miami, Lund, and Detroit, using incomplete forebrain ischemia models in rats, found that mild temperature changes can influence histologic outcome and deleterious mechanisms [55]. In recent years, this was taken to clinical trials in Europe [69], Australia [70], and Japan [71]—all with positive outcome results, in spite of late induction of mild hypothermia. In contrast to moderate hypothermia, mild hypothermia proved to be simple and safe. After cardiac

arrest, explorations of mild resuscitative hypothermia have begun for brain trauma, stroke, and other emergencies.

2. Conclusions

Modern CPCR was *inspired* by traumatology, *conceived* by steps A and B, *born* through step C and defibrillation, made *viable* with the addition of advanced and

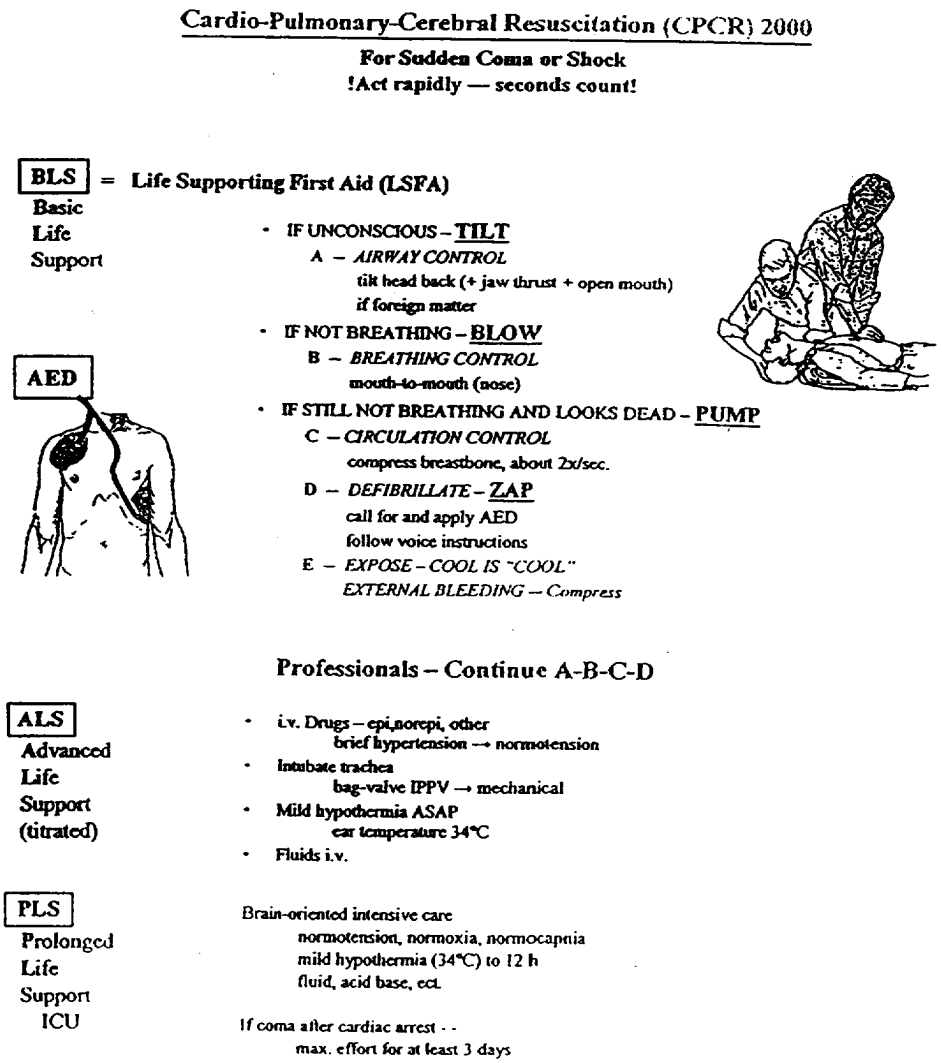

Fig. 2. The CPCR system proposed by Safar in 2000. BLS became LSFA, ALS and PLS, originally by the Pittsburgh group, became titrated and individualized, evidence-based.

prolonged life-support, and is now *maturing* with cerebral resuscitation. Cooling has become the hottest subject in resuscitation research. Industries have become interested in novel methods for rapid cooling.

How much have we learned? The knowledge about CPR that existed around 1900 was not implemented for 50 years. In 1960, steps A–B–C were accepted rapidly worldwide. In 2000, unfortunately, documented beneficial physiologic facts are still not implemented—but for reasons other than these we blame for failure in 1900. Avoiding another implementation gap now will require not letting agencies, committees, lawyers, conservatism, and insistence on positive "randomized clinical trials" prevail over physiologic facts.

What about presently unresuscitable acute death? Since 1984, Bellamy et al. [72], pondering over the pathophysiology of acute dying on battlefields, have recommended research into "suspended animation for delayed resuscitation" in presently unresuscitable exsanguination cardiac arrest. Suspended animation has been researched since the late 1980s in dogs, first with cardiopulmonary bypass [73], and recently by flushing the aorta with a very cold solution until brain temperature is around 10 °C [74]. The results of profound hypothermic preservation have surpassed anything achievable with drugs. Acute preservation of the organism in dogs during clinical death of 1–2 h proved possible [74].

The greatest resuscitation potentials in the near future will not lie in more modifications of already effective BLS, ALS, and PLS, but rather in research and development at the two extremes of the life-support chain. First, LSFA skills should be acquired by all fit humans, starting in elementary school [41]. It should include more emphasis on airway control with head tilt, automatic external defibrillation, and early initiation of cooling. This requires an upgrading of steps A–B–C of the CPCR system of 1961 (Fig. 1) to a proposed LSFA (i.e., BLS) and ALS–PLS system of 2000 (Fig. 2). Second, research for ultra-advanced life-support for presently unresuscitable traumatic and non-traumatic cases [72–74], perhaps to include "suspended animation for delayed resuscitation," should be supported and promoted, from the animal ICU via devices development, to clinical trials and use.

Resuscitation has created *ethical dilemmas* [75] that call for ongoing dialogue. The meaning of resuscitation medicine is to give more and more individuals a chance for a complete life span with "mens sana in corporae sano." Although resuscitation will save fewer people than will public health, the moral impact of resuscitation medicine affects many, in a world where life is often regarded as cheap. The 20th century has taught that, if practiced with reason and compassion, medicine in general and resuscitation medicine in particular, by constituting a higher ethic than chance, represent a positive force in human evolution.

Acknowledgements

The author's research has been supported by the A.S. Laerdal Foundation, the US National Institutes of Health, and the US Department of Defense. Patricia Boyle helped with editing. Fran Mistrick and Valerie Sabo helped with preparation of the manuscript. Dr. Doris Cope presented this slide talk at the Symposium in Santiago, Spain, since the author was unable to attend.

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EDITORIAL REVIEW

Trauma resuscitation: what have we learned in the last 50 years?

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Current Opinion in Anaesthesiology 2003, 16:133–138

Abbreviation

TBI traumatic brain injury

0952-7907

Introduction

During the past 50 years, i.e. during and since World War II, we have learned much theory about the pathophysiological mechanisms of traumatic hemorrhagic shock, analgesia, anesthesia, cytokine reactions, coagulopathy, wound infection, sepsis, wound healing, and neuropathophysiology concerning cardiac arrest and severe traumatic brain injury (TBI). We have learned that blood volume and oxygen delivery are more important than the type of resuscitation fluid. All of the foregoing represent much progress of scientific importance. Resuscitation methods have been given new technological advances for airway control, vessel access, and fluid resuscitation. These technological additions represent some progress of clinical importance. Still lacking are breakthroughs that would significantly increase the rates of intact survival among trauma patients, which would be progress of socioeconomic importance. In terms of decreasing the total number of deaths from injuries, prevention has been critical. The use of seat belts, air bags, helmets for motorcycle and bicycle use, and restraining seats for children have had important positive effects. In the United States, however, the programmes aimed at gun control and substance abuse are still inadequate.

Present resuscitation methods used in cases of severe polytrauma are based on the work of our predecessors during the first half of the 20th century. We owe much to the Anglo-American anesthesiologists and trauma surgeons of World War II. The major breakthroughs in knowledge and implementation came before and during World War II, primarily as a result of physiological studies on hypovolemic shock (by Crile, Wiggers, Guyton, Blalock, and others), blood banking and transfusion, endotracheal anesthesia (by Waters, Macintosh, and others), vaccination against tetanus, and antibiotics. Sulfonamides were introduced by German physicians before World War II, and penicillin was introduced by Anglo-American physicians at the end of the War.

How much progress has been made since World War II? The answer depends on how one measures progress. Mortality rates would give definitive answers, but only if one compares patients who had the same type of trauma and the same degree of deterioration at the moment they entered the life-support system. Such is rarely the case, and such data do not exist. When looking for documentation of increased intact survival rates with modern life support in trauma victims, we find convincing data only for TBI and burns. Although we agree in general with the appraisal of current trauma resuscitation by Gillham and Parr [1], we will here add some perspectives.

Much of the apparent progress has been the result of packaging old methods into systems, such as cardiopulmonary–cerebral resuscitation in the 1960s [2], and advanced trauma life support in the 1980s [3], and delivering them through community-wide emergency medical service organizations [4–6]. Basic trauma life support, i.e. life-supporting first aid by lay bystanders [7], has been promoted since the 1960s, but lack of control of the airway and of external hemorrhage by laypersons at the scene – the initial, most crucial steps – are still the weakest link in the life-support chain. Surgeons' emphasis until the 1950s on splinting and bandaging has wisely given way to anesthesiologists' emphasis on resuscitation [8]. New methods and devices have benefited orthopedic trauma cases, but fractures should take a backseat vis-à-vis resuscitation attempts.

Emergency medical services have to coordinate life support from the scene via transport to the most appropriate hospital's emergency department, operating room, and intensive care unit [5,6]. In some countries there has been regionalized centralization of the management of life-threatening trauma cases, particularly severe polytrauma and TBI with coma. This regionalization has increased the proportion of trauma patients having a good outcome [9]. Optimal care of the trauma patient requires a team approach including a variety of subspecialists. Only at regionalized trauma

DOI: 10.1097/01.aco.0000064031.76880.15

prescribed. For the use of plasma substitutes, the critical limits for normovolemic hemodilution were clarified in dogs in the 1960s [14].

Recently, debates have erupted about whether or not ambulance personnel should infuse a plasma substitute (and how much) before the patient is on the operating table. The answer was already simple in the 1940s, using common sense based on physiological facts: in cases with shock and controlled hemorrhage, the fluid infusion during and after hemostasis should aim for normotension and normovolemia. In cases of uncontrolled hemorrhage (common with penetrating truncal injuries), attempting to achieve normotension would be counterproductive (increase bleeding), as would also be the absolute withholding of fluid infusion, because cardiac arrest from severe hypovolemia can be sudden and is difficult to reverse. What level of controlled hypotension is safe? It has been known for the past 50 years that the achievement of arterial normotension does not signal the end-point for fluid volume expansion [13,15]. For in-hospital use, to decide when to stop fluid resuscitation after hemostasis, recently recommended monitoring methods have included such esoteric techniques as gastric tonometry and transcutaneous partial pressure oxygen or partial pressure of carbon dioxide, and super-normal oxygen delivery was considered. None was found to be more revealing than efforts to normalize blood lactate or base deficit values. The clinical monitoring of these values did not exist before the 1960s. Determining which patients have ongoing internal hemorrhage is not always easy. Response to an initial fluid bolus may help.

During the past 20 years, civilian trauma surgeons have found that less is frequently better [16–18]. This is particularly true in the most severely injured trauma victims, whose physiological reserve is depleted and a triad of hypothermia, coagulopathy, and acidosis portend a rapid spiral towards death. In the early 1990s, resuscitative surgery took on the terms 'damage control' and abbreviated or 'staged' celiotomy. These approaches have saved lives [16–18]. The concept is not new: it was used in as far back as 1908, and describes intrahepatic packing for severe liver injuries. The popularity of this manoeuvre waxed and waned until the late 1970s, when multiple reports suggested the benefits of perihepatic packing [17,18]. This same approach was soon applied for other injuries. Initially, this was often only used as a last resort, leading to poor outcomes. Surgeons believe that the phased approach has led to the better integration of fluid resuscitation and the restoration of homeostasis with the operative repair of injuries. Some trauma surgeons now try to anticipate the loss of physiological reserve and rapidly terminate the operative procedure. Whether this strategy will prevent coagulopathy and multiple organ failure, more surely than definitive repair, is debatable. With 'take-off and landing' in anesthesia management being risky, performing resuscitative and definitive operations as one procedure might make sense. Prolonged modern anesthesia, with the control of vital variables (titrated sedation, analgesia, and relaxation), i.e. 'pharmacologic hibernation' [19], might even increase survival chances in some cases. Therefore, whether definitive therapy beyond hemostasis should be delayed is a matter of individual judgement on a case-by-case basis.

For potentially exsanguinating hemorrhage in hospitals, the rapid massive administration of blood or plasma substitutes was occasionally practised over 50 years ago [20], using open bottles and burettes, rubber tubes, peripheral large-bore steel needles, air-pressure infusion and manual roller pumps. Blood warmers were introduced in the 1950s by anesthesiologists who discovered that cardiac arrest can occur during rapid intravenous infusion of large volumes of cold banked blood. Concerning exsanguinating hemorrhage, Negovsky *et al.* [21] studied massive infusion via an artery, and successfully used the method on combat casualties of World War II. Fifty years ago, some surprisingly good outcomes of massive intravenous blood infusions, without coagulopathies, were probably the result of the availability and wide use of type-specific fresh whole blood. The unavailability of fresh whole blood in our modern blood-banking system is a step backwards. Packed red cells and fresh frozen plasma, which take half-an-hour to be ready, are not the answer.

The Korean War experience increased our knowledge about acute tubular necrosis of the kidneys after hypovolemic shock of longer than 1 h duration (the golden hour). The Vietnam War experience increased our understanding of shock lung (so-called 'adult respiratory distress syndrome'). In Vietnam, the excessive intravenous loading with lactated Ringer's solution was a step backwards in relation to plasma and blood infusions in World War II and Korea.

Traumatic brain injury

The traumatized brain was a black box before the 1950s. Steps A and B of modern cardiopulmonary–cerebral resuscitation [2] and long-term intensive care life support [22], if started with airway control at the scene, can often achieve good results in comatose TBI patients. Many patients who once would have died before reaching the hospital are now resuscitated to survival – sometimes with and sometimes without subsequent permanent brain damage. Although mortality has been reduced, survivors with severe permanent brain damage have increased in numbers [23]. Early predictors of long-term outcome remain elusive, complicating rational decision making regarding when to withdraw support.

Besides optimal airway control from the moment of impact, and optimized life support of extracerebral organ variables, the introduction of intracranial pressure monitoring and control [24] has reduced mortality from severe TBI [23]. New knowledge acquired since the 1970s [25] concerning cerebral blood flow, metabolism, and perfusion pressure has helped develop rational life-support guidelines for TBI patients. Non-invasive roentgenological monitoring, particularly computed tomography and magnetic resonance imaging, has enabled the rapid accurate diagnosing of traumatic injuries throughout the body, and has proved particularly valuable for titrating brain-saving surgical and non-surgical therapies in a more rational manner. The vast and interesting ongoing research efforts to understand the molecular mechanisms behind post-TBI brain tissue damage and malfunction outside the grossly damaged areas, have not yet brought a therapeutic breakthrough. On hyperthermia, even 1°C above normal worsens TBI outcome. Resuscitative moderate hypothermia (30°C) was suggested for trauma cases as early as the 1950s [26]. It was revived for TBI in the 1990s with the confirmed benefit of mild hypothermia (33–36°C) in dogs [27,28] and patients [29]. Intracranial pressure normalization after TBI might require moderate hypothermia (30°C) [27,28]. Moderate hypothermia beyond 24 h may cause pulmonary infection [26,28]. Aggressive early brain rehabilitation may become an established factor in improved outcome.

Crushing chest injuries

Before the 1950s, some patients with severe blunt thorax trauma and flail chest wall asphyxiated. In the 1950s, 'internal stabilization' and oxygenation with prolonged controlled hyperventilation via tracheostomy tube was shown to turn the tide [30]. Recently, moderately severe cases were also saved without intratracheal strategies. Very severe cases have been saved using partial cardiopulmonary bypass (extracorporeal membrane oxygenation) [31].

Severe burns

With regard to burn care, the most important changes over the past 50 years have been in the areas of regionalized centralization of care, fluid resuscitation with mixtures of colloids and crystalloids, and wound management. Specialized burn centers are now the norm [32]. Although there continues to be controversy regarding the optimal fluid for the resuscitation of burn victims, it is clear that aggressive fluid resuscitation, while avoiding hypervolemia, is critical. Early excision of the burn wound, with coverage of some type has also improved outcomes. Finally, the early recognition of inhalation injuries with a low threshold for utilizing endotracheal intubation and ventilatory support has helped prevent early deaths from hypoxemia.

Combat casualties

The salvage rates in wars have been reduced only for the few patients who once would have 'died of wounds', from shock-related complications after they reach the hospital [33]. Combat casualties in hemorrhagic shock who, after initial all-out resuscitation, secondarily deteriorated into multiple organ failure and irreversible shock with septic components, were given up on in the past. In recent years, some have made it to complete recovery through sophisticated life support. The fate of those 'killed in action' has, however, apparently not changed since World War I [33,34]. Exsanguination cardiac arrest out-of-hospital has been considered to be unresuscitable. In agonal states, the intra-arterial infusion of oxygenated blood with epinephrine resulted in dramatic effects in dogs [21,35] and combat casualties [21]. The main obstacle remains the under-appreciation of the time factors involved and the fact that external cardiac massage is useless without intravascular volume and hemostasis. Therefore, research conceived by Safar and Bellamy [33] began in the late 1980s in dog outcome models of a new approach, i.e. 'suspended animation' (rapid preservation of the viability of the organism) for transport and hemostasis during clinical death, followed by delayed resuscitation [33,35,36]. These studies will lead to clinical feasibility trials in the near future.

The future

The future depends on the importance of resuscitation research results [35–41]. Ongoing resuscitation research promises to reveal several potentially futuristic breakthroughs. Hemostasis for uncontrollable internal hemorrhage might be achieved by non-invasive means. A futuristic attempt now under investigation uses high-frequency ultrasound in attempts to stop internal arterial hemorrhage. Hemostatic sponges and fibrin foam are also under development. A fresh look at the anti-shock trousers idea seems to be worthwhile. Pathophysiological studies on patients have provided much insight [37,38]. Randomized clinical outcome studies of traumatological resuscitation are plagued by the fact that each case has its own pathophysiology and time factors cannot be controlled. Clinical studies are needed to examine the feasibility and side-effects of new treatments. We ask whether it is ethical to randomly withhold novel treatments that have improved long-term outcomes in clinically relevant large animal models. The main obstacles to clinical outcome studies of trauma resuscitation are: the lack of control over pre-hospital care; the difficulty in getting trauma surgeons from many centers to agree on a protocol; the lack of readily available funding (except from industry); and the prospective consent requirement. Outcome evaluation in sequential trials should be considered. The introduction of national trauma case registries and an Utstein-type universally accepted method for evaluating clinical results would be

desirable. For TBI, one important field for future research will be a search for 100% reliable pathophysiological prognosticating measurements in severely brain-traumatized patients to facilitate early decision making on whether to let them die, to thereby significantly reduce suffering and cost.

The seemingly deleterious effects of uncontrolled hypothermia in trauma patients versus the benefits of controlled mild hypothermia in hemorrhagic shock outcome models in rats [37] will soon be clarified. For example, the Pittsburgh group [37], using new rat outcome models, challenged the critical 1 h limit of hemorrhagic shock tolerance by extending survival times and rates with mild preservative–resuscitative hypothermia. Mild hypothermia (33–36°C) does not seem to induce coagulopathy, arrhythmias, or infection (known to be caused by lower temperatures). Research is required to optimize the titration of the level and timing of hypothermia for prolonged hemorrhagic shock, and particularly for subsequent severe sepsis with multiple organ failure. The Pittsburgh group has found peritoneal strategies for oxygenation, medication, and cooling to look promising in rats. For suspended animation for delayed resuscitation [33,35,36], see above.

In uncontrolled hemorrhagic shock, development is needed for a titrated limited (hypotensive) infusion of a still-to-be-optimized novel blood substitute [38]. A bolus infusion of hypertonic saline/colloid and the use of hemoglobin-based oxygen carriers have not yet brought a breakthrough. Oxygen-carrying blood substitutes are sought for immediate availability, storage at ambient temperature, no disease transmission, and no need for cross-matching. Resuscitation fluids should be titrated and may well begin with the titrated use of a hypertonic/hyperoncotic small-volume infusion to mobilize extravascular fluid, and may continue with an isotonic or mildly hypertonic oxygen-carrying and medicated blood substitute titrated against other still-to-be-determined variables. Innovators should separate the dosing of novel drugs (e.g. ethyl pyruvate, adenosine, antioxidant tempol) from volume and oxygen delivery treatments.

Researchers should consider how to manage coagulopathies as a result of tissue trauma, hemodilution, ischemia, hypothermia, cardiopulmonary bypass, and reoxygenation injury. For preventing or treating coagulopathies in the meantime, a return to the use of group-specific, cross-matched fresh whole blood might be an answer. Plasma exchange and activated factor VII C look promising.

Future developments should range from innovative delivery systems, perhaps including the use of robots [41], to pathophysiology at the molecular level. Philosophical–physiological challenges for future research in trauma resuscitation include the timing and improved methods for supporting nature's initial 'flight and fight' response with a catecholamine surge, designed to prevent cardiac arrest, in contrast to the subsequent possibly beneficial and still-to-be-developed prolonged suppression of deleterious reflexes and chemical changes through 'hibernation which has various definitions' [19,42]. The latter might bring a breakthrough for sustaining the viability of vital organs during very prolonged traumatic hypovolemic shock states in casualties waiting for evacuation.

Conclusion

The past 50 years have brought traumatological resuscitation much new knowledge of pathophysiology, the wider, earlier, and better application of existing knowledge, and intriguing new technologies. All of this, regrettably, has led to only small increments in life-saving statistics. The real breakthroughs are still to come.

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clinical training in areas such as renal replacement therapy, transesophageal echocardiography, or bronchoscopy.

A preliminary analysis of a critical care fellowship survey being conducted by Dr. Gunnerson and Dr. Huang shows that 40% are willing to accept emergency physicians. This represents an increase from the response in a 1999 survey.[5] As more opportunities materialize, the influx of emergency physicians to the house of critical care will benefit the respective specialties, as well as critically ill patients. We encourage all interested emergency medicine residents to pursue advanced training in critical care, in whatever program they feel best meets their individual career goals.

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doi:10.1067/mem.2003.223

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In reply:

We are happy that our article has prompted comments from people as well respected as Dr. Rogers, Dr. Grenvik, and Dr. Fink. We are, of course, very familiar with the multidisciplinary model at the University of Pittsburgh. Many critical care physicians nationwide would long to work in an academic Department of Critical Care. We also recognize that their fellowship program accepts residency-trained emergency physicians as fellows. There are some differences, however, between their program and ours.

The program at the University of Pittsburgh accepts applications from emergency physicians. We have 4 slots dedicated only to emergency physicians. We believe this aspect is unique to any critical care fellowship. We do not fill those slots if we do not have suitable applicants. Their fellowship is a combination of medical and surgical rotations. Ours is a purely surgical critical care training program. Our emergency medicine fellows have the opportunity to be leaders on a high-volume trauma team, which we also believe is unique to our fellowship. The volume at the R Adams Cowley Shock Trauma Center allows both surgical and emergency medicine fellows to develop significant experience in the resuscitation and ongoing care of critically injured patients, without compromising resident education.

It is our opinion that, in the future, there will be a cadre of physicians whose academic and patient care mission will be to provide care to critically ill and injured patients. This physician pool will include surgeons, anesthesiologists, internists, and emergency physicians. The scope of critical care practice may well be broader than in the past. There is a real shortage of physicians necessary to provide the myriad of services necessary to provide comprehensive care to critically ill and injured patients. For instance, anesthesiologists and interventional radiologists are currently in short supply. We believe that the intensivist may well provide more comprehensive care in the future.

Critical care training for emergency physicians makes absolute sense. We embrace all of the sentiments articulated by the group from the University of Pittsburgh and fervently hope that many other critical care training programs will not only accept emergency physicians, but dedicate spots to them.

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doi:10.1067/mem.2003.224

Mild Hypothermia in Resuscitation: A Historical Perspective

To the Editor:

The interesting review article on therapeutic mild hypothermia by Inamasu and Ichikizaki[1] (August 2002; article #123697) correctly points out many therapeutic potentials. I would like to clarify some historic features of hypothermia research.

Protective-preservative hypothermia (during the insult) was pioneered in the 1950s.[2] Resuscitative hypothermia (after the insult) lay dormant between the 1960s and 1980s, probably because of management difficulties, arrhythmias, coagulopathy, and infection associated with moderate hypothermia (28°C to 32°C [82.4°F to 89.6°F]), a level then believed necessary for hypothermia to

be beneficial. The recent revival of therapeutic hypothermia was born of trials, not for traumatic brain injury, but rather for cardiac arrest. In the early 1980s, the University of Pittsburgh group (Brader, Gisvold, Leonov, and Safar), disappointed with drug trials for cerebral resuscitation after cardiac arrest, revived research into moderate hypothermia in dogs.[3] The benefit achieved was modest. In 1987, at a meeting of clinical-death-oriented researchers in Pittsburgh, PA, Hossmann[4] presented improved electroencephalogram recovery in cats after global brain ischemia when mild hypothermia had occurred accidentally; and Safar[5] discovered benefit from preservative (intra-ischemic) accidental mild hypothermia (33°C to 36°C [91.4°F to 96.8°F]) in post–cardiac arrest outcome data in dogs. This was followed between 1988 and 1994 by 5 outcome studies in dogs that documented, for the first time after normothermic cardiac arrest, no-flow of 10 to 12 minutes and, with long-term intensive care, the ability of mild resuscitative (postischemic) hypothermia (which is simple and safe) to reduce brain damage[6-8] and to normalize functional and histologic outcome after 11-minute cardiac arrest.[8] The latter is important because average urban mobile ICU ambulance response times are approximately 8 minutes. Simultaneously and independently, researchers in Miami, FL,[9] Lund, Sweden,[10] and Detroit, MI,[11] found mild hypothermia to mitigate histologic damage and various deleterious mechanisms in rodent models of cerebral ischemia. It was the dog outcome data[6-8] that led to the positive randomized clinical outcome studies in Europe and Australia published in *The New England Journal of Medicine* in February 2002.[12-14] Cardiac arrest intensive care outcome studies in dogs are rarely quoted, although they are clinically more realistic than studies in rodents and scientifically more controllable than randomized clinical trials. Only after the documentation of mild resuscitative hypothermia effects on outcome after prolonged normothermic cardiac arrest[6-8] was the revival of hypothermic strategies adopted by traumatic brain injury researchers. The first positive clinical study of mild hypothermia after traumatic brain injury, by Marion et al,[15] was preceded by a positive outcome study in dogs of traumatic brain injury simulation with temporary epidural brain compression,[16] which showed that control of intracranial pressure in traumatic brain injury may sometimes require moderate (not just mild) levels of hypothermia.

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doi:10.1067/mem.2003.215

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In reply:

My fellow author and I sincerely thank Dr. Safar for providing important and informative commentary on our article.

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doi:10.1067/mem.2003.216

Hopelessly Complex

To the Editor:

"Hopelessly Complex" is the title of the lecture that I give to our residents about documentation guidelines. The article (#123692) by Bentley et al[1] in the September 2002 issue of *Annals* highlights this problem. I agree with their concern that it is unfair for the Office of Inspector General to prosecute providers for billing infractions unless they can show consistency in chart reviews. However, the current

Survival without brain damage after clinical death of 60–120 mins in dogs using suspended animation by profound hypothermia*

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Objectives: This study explored the limits of good outcome of brain and organism achievable after cardiac arrest (no blood flow) of 60–120 mins, with preservation (suspended animation) induced immediately after the start of exsanguination cardiac arrest.

Design: Prospective experimental comparison of three arrest times, without randomization.

Setting: University research laboratory.

Subjects: Twenty-seven custom-bred hunting dogs (17–25 kg).

Interventions: Dogs were exsanguinated over 5 mins to cardiac arrest no-flow of 60 mins, 90 mins, or 120 mins. At 2 mins of cardiac arrest, the dogs received, via a balloon-tipped catheter, an aortic flush of isotonic saline at 2°C (at a rate of 1 L/min), until tympanic temperature reached 20°C (for 60 mins of cardiac arrest), 15°C (for 60 mins of cardiac arrest), or 10°C (for 60, 90, or 120 mins of cardiac arrest). Resuscitation was by closed-chest cardiopulmonary bypass, postcardiac arrest mild hypothermia (tympanic temperature 34°C) to 12 hrs, controlled ventilation to 20 hrs, and intensive care to 72 hrs.

Measurements and Main Results: We assessed overall performance categories (OPC 1, normal; 2, moderate disability; 3, severe disability; 4, coma; 5, death), neurologic deficit scores (NDS 0–10%, normal; 100%, brain death), regional and total brain histologic damage scores at 72 hrs (total HDS >0–40, mild; 40–100, moderate; >100, severe damage), and morphologic damage of extracerebral organs. For 60 mins of cardiac arrest (n = 14), tympanic temperature 20°C (n = 6) was achieved after flush of 3 mins and resulted in two dogs with OPC 1 and four dogs with OPC 2: median NDS, 13% (range 0–27%); and median total HDS, 28 (range, 4–36). Tympanic temperature of 15°C (n = 5) was achieved after flush of 7 mins and resulted in all five dogs with OPC 1, NDS 0% (0–3%), and HDS 8 (0–48). Tympanic temperature 10°C (n = 3) was achieved after flush of 11 mins and resulted in all three dogs with OPC 1, NDS 0%, and HDS 16 (2–18). For 90 mins of cardiac arrest (n = 6), tympanic temperature 10°C was achieved after flush of 15 mins and resulted in all six dogs with OPC 1, NDS 0%, and HDS 8 (0–37). For 120 mins of cardiac arrest (n = 7), three dogs had to be excluded. In the four dogs within protocol, tympanic temperature 10°C was achieved after flush of 15 mins. This resulted in one dog with OPC 1, NDS 0%, and total HDS 14; one with OPC 1, NDS 6%, and total HDS 20; one with OPC 2, NDS 13%, and total HDS 10; and one with OPC 3, NDS 39%, and total HDS 22.

Conclusions: In a systematic series of studies in dogs, the rapid induction of profound cerebral hypothermia (tympanic temperature 10°C) by aortic flush of cold saline immediately after the start of exsanguination cardiac arrest—which rarely can be resuscitated effectively with current methods—can achieve survival without functional or histologic brain damage, after cardiac arrest no-flow of 60 or 90 mins and possibly 120 mins. The use of additional preservation strategies should be pursued in the 120-min arrest model. (Crit Care Med 2003; 31:1523–1531)

KEY WORDS: cardiac arrest; hypothermia; hemorrhage; resuscitation; cerebral ischemia; cardiopulmonary bypass

In considering resuscitation from severe hemorrhage, one must differentiate between hemorrhagic shock, which is low-flow and common, and exsanguination cardiac arrest (CA), which is no-flow and rare. CA is the topic of this study. Civilian trauma patients and military combat casualties with penetrating (often repairable) trunkal injuries exsanguinate rapidly to CA. Conventional resuscitation attempts are futile, and survival rates are near zero (1–4). For such unresuscitable conditions, since 1984, Safar and Bellamy have recommended research into "suspended animation for delayed resuscitation." This they have defined as "induction of preservation of the organism within the first 5 min of CA (no-flow) for transport and surgical hemostasis during clinical death, to be followed by delayed resuscitation to survival without brain damage" (4).

Treatment induced before arrest (protection) and maintained during arrest (preservation) is more likely to mitigate postischemic brain damage than when induced after arrest (resuscitation) (5, 6). Suspended animation is preservation-resuscitation with use of drugs or hypothermia. Using systematic studies of exsanguination CA in a reproducible dog outcome model with induction of preservation by aortic flush at 2 mins CA, of saline at 24°C, via a balloon-tipped catheter (7–10), we obtained disappointing

*See also p. 1592.

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Supported, in part, by the U.S. Department of Defense, Office of Naval Research, grant N00014-97-1-1064; by the U.S. Army's MRMC/TATRC, grant N00014-99-1-0765; and by the Cardeon Corporation, which provided the flush catheter.

DOI: 10.1097/01.CCM.0000063450.73967.40

outcome results with a series of mechanism-specific pharmacologic therapies (11–15). The exception was the antioxidant tempol, which improved functional outcome (15). In contrast, lowering the temperature of the flushed saline to 2°C and progressively increasing the flush volume, starting the flush at 2 mins of normothermic exsanguination CA, we could decrease brain (tympanic membrane) temperature (Tty) to around 34°C, which preserved brain viability during CA of 15 mins (7) and 20 mins (8), and to around 28°C, which preserved brain viability for 30 mins (9). The present study is an extension of these systematic efforts to maximize the duration of CA (no-flow) from which resuscitation to survival can be achieved without vital organ system damage (10). Attempting to extend this maximal CA period from 30 to 120 mins is called for by the fact that transport and surgical hemostasis in patients with traumatic exsanguination to CA would require such prolonged preservation, particularly in military combat scenarios.

Protective-preservative hypothermia, induced and reversed with cardiopulmonary bypass (CPB), is clinically used for some elective operations on heart or brain but has not been evaluated yet for emergency scenarios as in this study. Elective therapeutic hypothermia has been shown to protect the brain and whole organism in animals or patients for up to 15 mins of CA at brain temperature of about 35°C (mild hypothermia) (16, 17), for up to 20 mins of CA at about 30°C (moderate hypothermia) (18), for up to 30 mins of CA at about 20°C (deep hypothermia) (19), for up to 60–150 mins of CA at 5–10°C (profound hypothermia) (20–27), and perhaps even for longer CA with ultraprofound hypothermia (28–30). The normal brain is not damaged by temperatures lowered to 5–10°C (31) but can be damaged by temperatures below 5°C (32, 33). In most of the previously mentioned studies of protective-preservative hypothermia for elective prolonged CA (23–30), induction of hypothermia was with CPB, before induction of CA and without total exsanguination; also, evaluation of cerebral function and histology was not quantitative as in our present study.

The experiments reported in this article are the first systematic explorations of emergency measures aimed at maximizing the reversible CA no-flow duration. The method should ultimately be inducible for patients also outside hospitals. Ours is the only group experimenting with this suspended animation approach, which includes early and rapid induction of preservation with aortic flush and intensive care life support for 72 hrs to give the ischemic anoxic encephalopathy time to mature. The objective of this study was to simulate the scenario of rapid exsanguination to death from a laceration in the aorta or vena cava and to determine, for the first time, the longest no-flow period from which resuscitation to complete recovery can be accomplished with the aid of CPB. This study is also the first to use a single aortic saline flush immediately after the start of CA (no-flow), to include preservation and long-term intensive care to outcome evaluation in terms of function and semiquantitative histologic brain damage. We hypothesized (10) that preservation during CA 60, 90, or 120 mins (no-flow) requires Tty 10°C to achieve intact survival without histologic brain damage.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and the Department of Defense and followed national guidelines for the treatment of animals. All experiments were conducted by the same team between May 2000 and January 2001, in mixed sequence, without randomization. The protocol called for exsanguination to CA: in study A with 60 mins of no-flow, comparing three preservative levels of hypothermia (Tty 20, 15, or 10°C), and in study B with 90 mins vs. 120 mins of no-flow at Tty 10°C. Resuscitation was with CPB, mild hypothermia to 12 hrs, controlled ventilation to 20 hrs, and intensive care to final outcome evaluation at 72 hrs. At CA 2 mins, the dogs received a flush into the aorta with saline at 2°C, in study A until Tty reached 20°C (n = 6), 15°C (n = 5), or 10°C (n = 3) for CA 60 mins, and in study B until Tty reached 10°C for CA 90 mins (n = 6) or 120 mins (n = 7, of which 3 had to be excluded, as discussed in the Results).

Preparation. We studied 27 custom-bred hunting dogs (17–25 kg body weight, age 8–12 months, simulating young healthy trauma victims). Details of preparation and model have been described (7–9, 13–15). Briefly, after premedication with ketamine, orotracheal intubation, and anesthesia with halothane and N_2O/oxygen (1:1), temperature probes were inserted for measuring Tty and esophageal (Tes) and rectal temperatures (Tr). In pilot experiments during flush, brain tissue temperature decreased more rapidly than Tty, but the two equilibrated rapidly within ±1°C. Intravenous maintenance fluid was with dextrose 5% in 0.45% NaCl. The left femoral artery was cannulated for monitoring of arterial pressure. A pulmonary artery catheter was inserted to monitor pressure, cardiac output, and temperature (Tpa). A prototype balloon catheter (8 Fr), with one hole at the tip of the catheter, was advanced via the right femoral artery into the aorta for arterial bleeding and for the aortic flush. The right external jugular vein was cannulated with a multiple-holed cannula (18 Fr), which was advanced to the level of the right atrium for venous bleeding and for venous return to the CPB system. Arterial and central venous pressures and the electrocardiogram were continuously recorded. Arterial and mixed venous blood gases, hemoglobin, hematocrit, sodium, potassium, glucose, and lactate were measured at regular intervals. Blood gases were controlled as measured at normothermia (alpha-stat strategies). Samples for liver function (glutamyl oxaloacetic transaminase, γ-glutamyl transpeptidase, and bilirubin in serum) and kidney function (creatinine in serum and urine) were taken at baseline, 24 hrs, and 72 hrs. Just before start of the insult, Tty was controlled at 37.5 ± 0.1°C by heating blanket and lamp.

Insult. After two baseline measurements, heating devices, intravenous fluids, and halothane were discontinued, while the dogs were weaned to spontaneous breathing of N_2O/oxygen (2:1) via a T-tube. When the canthal reflex returned (as an indication of very light anesthesia), hemorrhage was initiated. Over a 5-min period, the dogs were bled via the arterial and venous cannulae (simulating traumatic laceration), and the blood was collected in bags with sodium citrate anticoagulant for later reinfusion. Hemorrhage was controlled to mean arterial pressure (MAP) 20 mm Hg at 4 mins. At 5 mins, to ensure zero blood flow, ventricular fibrillation was induced with one or more subcutaneous transthoracic shocks of 110 V AC, to fully control the onset of circulatory arrest. Total arrest (no-flow) time was 60 mins in study A and 90 or 120 mins in study B.

Aortic Flush. Two minutes after the onset of CA, the balloon of the aortic catheter, placed in the abdominal aorta, was inflated with 1.5 mL of saline, known to occlude the aorta (9). Saline at 2°C then was flushed into the aorta at a rate of 1 L/min by using a roller pump (care was taken to avoid air entering the system). In study A, the flush was stopped when Tty reached 20°C (n = 6), 15°C (n = 5), or 10°C (n = 3). In study B, for CA 90 mins, the flush was stopped when Tty reached 10°C (n = 6). For CA 120 mins, a pilot experiment had shown the hind legs with rigor mortis at start of resuscitation, and the rectum was necrotic at necroscopy. Therefore, in study B, for CA 120 mins, the balloon was first placed in the thoracic aorta until Tty reached 10°C and then deflated and pulled back into the femoral artery, continuing the flush until Tr reached 20°C (n = 7). After the flush, during CA, the

aortic catheter was replaced with a short arterial CPB cannula (7 or 8 Fr).

During CA, the head and neck were immersed in ice water to prevent the spontaneous rewarming by 3–5°C we have seen previously during CA of 60–120 mins without ice water immersion. In two pilot experiments, laryngeal-pharyngeal edema occurred after the whole neck was immersed in ice water >1 hr; therefore, in study B only the head was immersed in ice water and neck edema did not occur.

Resuscitation. After CA no-flow of 60, 90, or 120 mins, reperfusion was with CPB, because standard cardiopulmonary resuscitation cannot reliably achieve restoration of spontaneous circulation (ROSC) from CA >12 mins of no-flow (6, 16, 17). The CPB circuit was primed with 400 mL of Dextran 40 10% plus Ringer's solution (1:1). Sodium bicarbonate (2 mEq/kg) and heparin (1500 units) were added. Just before the start of CPB, additional sodium bicarbonate (1 mEq/kg) was injected into the circuit. The dogs were paralyzed with pancuronium (0.1 mg/kg intravenously). The temperature of the water bath of the CPB heat exchanger was set to 5°C above Tty, until Tty reached 34°C. CPB was started with a flow of 50 $mL \cdot kg^{-1} \cdot min^{-1}$ for Tty <20°C, increased to 75 $mL \cdot kg^{-1} \cdot min^{-1}$ for Tty 21°C–30°C, and increased to 100 $mL \cdot kg^{-1} \cdot min^{-1}$ for Tty >30°C. Reinfusion of all shed blood was titrated to achieve a central venous pressure of 10–15 mm Hg. Repetitive doses of epinephrine (0.01 mg/kg) were given intra-arterially as necessary to increase MAP to 60 mm Hg during Tty <20°C, to 80 mm Hg during Tty 21–30°C, and to 100 mm Hg during Tty >30°C. When Tpa reached 32°C, defibrillation attempts were with external DC countershocks of 150 J, increased by 50 J for repeated shocks. Oxygen flow through the oxygenator was adjusted to keep $Paco_2$ at 30–35 mm Hg and Pao_2 ≥100 mm Hg. During CPB of 2 hrs, controlled ventilation was with 100% oxygen at a rate of eight to ten inflations per minute. The intravenous fluids were restarted with a flow of 100 mL/hr. A base deficit of >6.0 mEq/L was corrected with sodium bicarbonate. When ROSC was established, a norepinephrine infusion was titrated intravenously to achieve a brief hypertension of MAP ≥150 mm Hg (9). We include in standard protocols hypertensive reperfusion, which improves cerebral blood flow and outcome (34). Thereafter, MAP was controlled at 90–150 mm Hg. The CPB flow rate for assisted circulation was reduced to 75 and 50 $mL \cdot kg^{-1} \cdot min^{-1}$ and stopped at 120 mins. During CPB, activated clotting times were maintained at >300 secs with additional heparin as needed.

Intensive Care. After weaning from CPB assist at 2 hrs, controlled ventilation was continued to 20 hrs with N_2O/oxygen 1:1 for analgesia. Paralysis was maintained with intermittent doses of pancuronium. To prevent stress, fentanyl boluses (5–10 μg/kg) were given intravenously whenever signs of possible distress (mydriasis, tachycardia, or hypertension) occurred. In pilot experiments without paralysis, there were no escape movements with this regimen (9). Hypotension (MAP <90 mm Hg) was treated with titrated intravenous infusion of Ringer's solution or norepinephrine. Severe hypertension (MAP >150 mm Hg) was controlled with titrated intravenous boluses of labetalol or hydralazine. Standard intensive care included airway suctioning, periodic deep lung inflations, and position change (rotation). The dogs received cefazolin every 8 hrs for infection prophylaxis. At 20–24 hrs, paralysis was reversed to spontaneous breathing with neostigmine plus atropine. The dogs were extubated when they were able to maintain normal blood gas values during spontaneous breathing and after upper airway reflexes had returned. The catheters were then removed under brief, light N_2O-halothane anesthesia by mask. When awake and stable, the dog was transferred to a stepdown intensive care unit to 72 hrs, with oxygen by mask, continuous monitoring of pulse rate, and arterial oxygen saturation by technicians and critical care physicians. Seizures, running movements, opisthotonos, or spontaneous tachypnea were controlled with titrated doses of diazepam (0.2–0.3 mg/kg intravenously) as needed. Tty was controlled at 34°C with external cooling and warming for the first 12 hrs after start of CPB and at 37.5°C until 72 hrs. The maintenance intravenous fluid was dextrose 5% in NaCl 0.45% until 24 hrs and dextrose 10% in NaCl 0.45% thereafter for supply of energy—until the dog was able to eat and drink. Optimal blood glucose concentrations after prolonged CA are unknown (35, 36).

Outcome Evaluation. Function and cerebral morphologic changes were evaluated as described before (6, 34, 37–40). Briefly, performance was evaluated according to overall performance categories (OPC 1, normal; 2, moderate disability; 3, severe disability; 4, coma; and 5, death or brain death). Neurologic function was evaluated as neurologic deficit scores (NDS 0–10%, normal; 100% = brain death). OPC and NDS were evaluated every 8 hrs after extubation. Final evaluations (72 hrs) were independently determined and agreed upon by two team members. Attempts were made to discontinue any sedation ≥4 hrs before final evaluations. If necessary, sedation was reversed with flumazenil.

After final outcome evaluation at 72 hrs, for morphologic studies (37), the dogs were reanesthetized as before, the left hemithorax was opened, and the proximal descending aorta was ligated. A large-bore cannula was inserted proximal to the ligature. The dogs then were killed by infusing into the aortic arch approximately 2 L of paraformaldehyde (4%, pH 7.4). A complete necropsy was performed, and samples of extracerebral organs were taken for histologic examination. Macroscopic scoring was performed of damage in gut and heart (mild vs. moderate vs. severe hemorrhage, any necrosis).

One hour after perfusion fixation, the brain was removed. After 3-mm thick slices were cut, the same six slices of each brain were paraffin embedded, cut into sections 4 microns thick, and stained with hematoxylin-eosin-phloxine (37). Using light microscopy, the same pathologist (AR), unaware of treatment, group assignments, and hypotheses, scored 19 distinct anatomical brain regions for severity and extent of ischemic neuronal changes (shrunken eosinophilic neurons with pyknotic nuclei), infarcts, and edema, as described previously (37, 38). The total brain histologic damage score (HDS) was the sum of all area scores. A total HDS between zero and about 40 has usually correlated with normal or minimally impaired function, about 40–100 represented moderate damage, and total HDS >100 indicated severe damage (37, 38). Extracerebral variables were monitored as described in the Results.

For exploration of cognitive function recovery, three dogs with OPC = 1 and NDS 0–10% (normal) at 72 hrs—one of study A after CA 60 mins at Tty 20°C, one of study B after CA 90 mins at Tty 10°C, and one normal dog without CA—were evaluated over 6 months for their ability to learn a spatial version of a successive reversal learning task, modified from Head et al. (41). The dogs were initially habituated to a test chamber. On one wall of the chamber were two head holes that could be covered or opened by a sliding door. Outside of each head hole was a block that covered the reward. The dogs were trained to find a food reward based on the spatial position (left vs. right) that was the reverse of the preceding trial. The reward was alternatively hidden under the left or right block and a correct response was counted if the dog chose the side with the reward. We measured the number of sessions, of ten consecutive trials each, needed to twice achieve eight of ten correct responses.

Statistical Analysis. Dogs that either did not meet protocol criteria or died from extracerebral causes before 72 hrs were excluded from outcome analysis. Brain death as an outcome was included if the study process satisfied protocol. Data are given as mean and SD, if normally distributed, and otherwise as median and range. This study was an exploratory one not depending on statistical group differences, and in study A, we did not perform any statistical group comparisons, since one group consisted of only three dogs. Merely for the purpose of complete information, we used in study B the independent samples *t*-test or the Mann-Whitney U test to compare continuous variables (physiologic variables, NDS, HDS) and the chi-square test for trend to test for differences in proportions of OPC values between groups. All data were computed with SPSS for Windows (release 8.0; Chicago, IL) or NCSS for Windows (UT). We considered $p < .05$ to be statistically significant.

RESULTS

For both studies, a total of 27 dogs were exsanguinated to CA. In study A with CA 60 mins (n = 14) and in study B with CA 90 mins (n = 6), all dogs survived to 72 hrs in protocol. In study B with CA 120 mins (n = 7), three dogs failed to survive to 72 hrs and had to be excluded because one developed pulmonary edema during CPB, hemorrhagic diarrhea, anemia, and hemorrhagic lungs and was killed at 23 hrs; one had the flush delayed due to technical pump error; and one died at 25 hrs unrecognized in the stepdown unit after premature extubation due to human error. Thus, only four of the seven dogs in the CA 120-min group survived to final evaluation at 72 hrs. One dog of study A after CA 60 mins at Tty 20°C, and one dog of study B after CA 90 mins at Tty 10°C, although within protocol, provided only functional outcome data because they were kept alive for cognitive function testing at 6 months, leaving 22 of the 27 dogs for morphologic evaluation at 72 hrs.

Heart rate, MAP, and arterial Po_2, Pco_2, hematocrit, base excess, sodium, potassium, glucose, and lactate—at baseline and at resuscitation time 6 hrs—were within normal ranges, except in study B at 6 hrs, where the blood glucose values in the CA 120-min group were statistically higher (median, 316 mg/dL; range, 289–363) than in the CA 90-min group (median, 227 mg/dL; range, 107–253; $p = .01$).

Flush and Temperatures. Flush volumes and durations needed to achieve the target Tty are given in Table 1. After we stopped the aortic flush, Tty decreased spontaneously slightly further, in study A with CA 60 mins to a lowest Tty of 18.3 ± 1.8°C (range, 15.1–20.0) in the 20°C group; to 14.4 ± 0.3°C (14.2–14.9) in the 15°C group; and to 10°C, 9.7°C, and 9.8°C in the 10°C group (Fig. 1*A*). In study B with CA 90 mins, the lowest Tty was 9.2 ± 1.0°C (7.5–10.0; Fig. 1*B*), and with CA 120 mins the lowest Tty was 6.9 ± 0.8°C (5.9–7.9; Fig. 1*B*). Lowest Tes (core T) ranged between 15.1 and 24.8°C in the Tty 20°C group, between 16.3°C and 22.8°C in the Tty 15°C group, and between 6.1 and 15.3°C in the Tty 10°C groups. Lowest Tr was approximately 35–37°C, except for the CA 120-min dogs (with cold flush into the femoral artery), in which lowest Tr ranged between 20.0 and 24.6°C. In one dog, the flush was stopped when 15 L flush volume was consumed, with Tr 24.6°C.

Resuscitation. During reperfusion with CPB, the time required to increase core temperature (Tpa) to 32°C depended on the depth of hypothermia at the end of CA (Table 1). In both studies, once Tpa reached 32°C, ROSC was achieved after one to three countershocks (Table 1). In study A, one dog in the 20°C group spontaneously developed QRS complexes at the start of CPB and ROSC at 5 mins after the start of CPB. The amount of epinephrine required to keep MAP in protocol during CPB did not differ between groups, nor did the amounts of norepinephrine and bicarbonate required after ROSC (Table 1). There were no significant group differences in the highest MAP and the duration of the brief induced hypertension, which ranged between 1 and 14 mins (Table 1).

Complications. In addition to the complications that caused three exclusions (described previously), there were complications in four of the included dogs: One of the five included dogs with CA 60 mins at 15°C developed increased pulmonary artery occlusion pressure and pulmonary edema while on controlled ventilation. The pulmonary edema was managed with increasing positive end-expiratory pressure; arterial Po_2 remained >200 mm Hg. One dog after CA 60 mins developed fever in the stepdown unit at 32 hrs, which was reversed with acetaminophen by mouth. One dog of this group had hemorrhagic diarrhea during the observation phase. One dog, after CA 90 mins at 10°C, had hemorrhagic diarrhea during the observation phase; one dog of the same group developed tachycardic atrial fibrillation 1 hr after the start of CPB, which was resistant to amiodarone and diltiazem but could be terminated with one synchronized countershock (50 J) at 24 hrs.

Overall and Cerebral Outcome. OPC, NDS, and total brain HDS at 72 hrs are shown in Figure 2; regional HDS is shown in Figure 3.

In study A, after CA 60 mins at Tty 20°C, two dogs achieved OPC 1 with NDS 0% and 1% (normal). In one of these dogs, total brain HDS was 28, and the other dog was kept for cognitive function testing. Four dogs achieved OPC 2 due to various degrees of motor impairment of the hind legs, including inability to stand and walk, which resulted in NDS 6–27%;

Table 1. Aortic flush volume and duration needed to achieve target tympanic temperature: Requirements for restoration of spontaneous circulation (ROSC) and hypertensive bout

	Study A (60 Mins CA)						Study B (Tty 10°C)			
	Tty 20°C (n = 6)		Tty 15°C (n = 5)		Tty 10°C (n = 3)		90 Mins (n = 6)		120 mins (n = 4)	
Flush volume, mL/kg	159	(144–228)	306	(258–373)	430, 469, 546		578	(535–736)	666	(598–755)
Flush duration, min:sec	3:30	(3:15–5:30)	7:10	(5:19–8:40)	9:11, 10:55, 12:15		14:33	(11:50–15:58)	15:17	(13:45–16:39)
Time to reach Tpa 32°C, min	22	(11–23)	20	(13–55)	31, 32, 33		44	(24–56)	43	(29–62)
Countershocks, total number	1	(0–2)	1	(1–3)	1, 1, 1		1	(1–1)	1	(1–2)
Countershocks, total energy, J	150	(0–300)	150	(150–500)	150, 150, 150		150	(150–150)	150	(150–300)
Total bicarbonate, mEq	205	(165–235)	185	(155–270)	200, 200, 230		220	(190–280)	250	(150–290)
Total epinephrine, mg	0.9	(0.2–2.4)	1.0	(0.2–1.8)	0.6, 1.0, 1.5		1.6	(0.4–3.4)	1.3	(1.1–1.8)
Total norepinephrine, mg	0.96	(0.80–1.76)	1.12	(0.96–2.24)	1.76, 1.92, 2.88		3.12	(0.8–7.68)	1.20	(1.12–1.28)
Hypertensive bout: peak MAP, mm Hg	175	(160–200)	165	(150–200)	150, 155, 160		165	(150–175)	165	(150–170)
Hypertensive bout start, min[a]	22	(9–29)	30	(19–63)	40, 41, 43		54	(28–64)	49	(36–67)
Hypertensive bout duration, min[b]	5	(3–7)	4	(3–5)	2, 4, 6		4	(1–14)	4	(3–4)

CA, cardiac arrest; Tty, tympanic temperature; Tpa, pulmonary artery temperature; MAP, mean arterial pressure.

[a]Start of hypertensive bout = time after start of cardiopulmonary bypass; [b]duration of hypertensive bout = time with MAP >150 mm Hg. Data are given as median (range) or as single values.

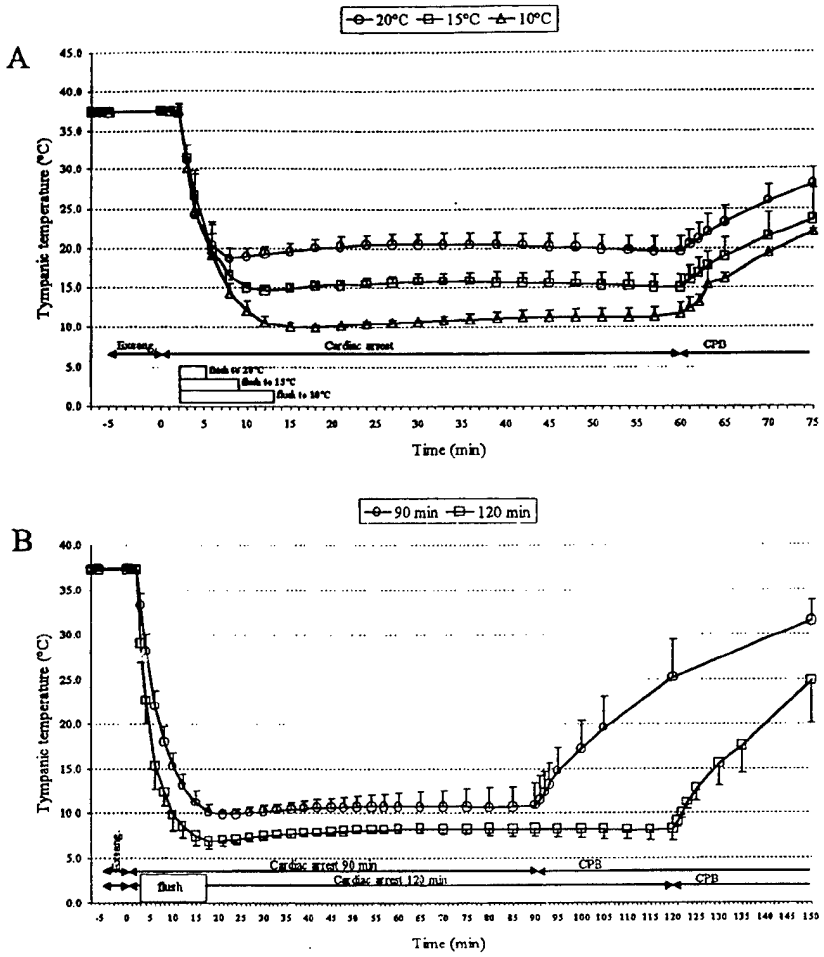

Figure 1. Tympanic membrane temperatures during exsanguination cardiac arrest of 60 mins of no-flow in study A (*A*) and 90–120 mins of no-flow in study B (*B*). Resuscitation was with cardiopulmonary bypass (*CPB*). Data are given as mean and SD.

	Study A (60 min CA)			Study B (Tty 10°C)	
	Tty 20°C (n = 6)	Tty 15°C (n = 5)	Tty 10°C (n = 3)	90 min CA (n = 6)	120 min CA (n = 4)
OPC 5 (death or brain death)					
OPC 4 (coma)					
OPC 3 (severe disability)					•
OPC 2 (moderate disability)	••••				•
OPC 1 (normal)	••	•••••	•••	••••••	••
NDS (%)	13 (0 – 27)	0 (0 – 3)	All 0	All 0	10 (0 – 39)
HDS	28 (4 – 36)*	8 (0 – 48)	2, 16, 18	8 (0 – 37)†	17 (10 – 22)

Figure 2. Outcome in terms of final overall performance categories (*OPC 1–5*) at 72 hrs after exsanguination cardiac arrest of 60 mins no-flow in study A and cardiac arrest of 90–120 mins no-flow in study B. Each dot represents one dog. *NDS*, neurologic deficit scores (1–100%); *HDS*, total brain histologic damage scores (0–40, no or mild damage; 40–100, moderate damage; >100, severe damage). *n = 4 and †n = 5 (one dog each was maintained for cognitive function testing).

they showed normal cerebral performance. Their total brain HDS was 4–36. Histologic evaluation of the spinal cord did not show any pathology. In the Tty 15°C and 10°C groups, all dogs achieved OPC 1 and NDS 0% (except that one in the 15°C group had NDS 3% due to weak hind legs). Total brain HDS was <40 in all dogs in the 15°C and 10°C groups, except one in the 15°C group with worse HDS. Histologically normal brains were seen in one dog in the Tty 20°C group (HDS 4), two dogs in the 15°C group (HDS 0 and 6), and one dog in the 10°C group (HDS 2).

In study B, after CA 90 mins at Tty 10°C (n = 6), all dogs were functionally normal (OPC 1 and NDS 0%), with total brain HDS = 8 (range, 0–37; n = 5). One dog had HDS zero. After CA 120 mins (n = 4), functional outcomes varied. One dog achieved OPC 1 with NDS 0% and total HDS 14. One dog achieved OPC 1 with NDS 6% due to mild disability in the hind legs and HDS 20. One dog achieved OPC 2 with NDS 13 due to weakened hind legs and HDS 10. One dog achieved OPC 3 (poor outcome) with NDS 39 and HDS 22. Total brain HDS were <40 in all dogs after CA 90 or 120 mins and also in the one dog with OPC 3.

Regional brain HDS showed the same distribution in all groups. Therefore, the results are summarized for all 22 dogs in which HDS was evaluated (Fig. 3). In 17 of the 19 regions, the median HDS was zero. Putamen and caudate nucleus seemed to be the most vulnerable regions in this model, as was the case in this model also with moderate hypothermia. All scores were the result of scattered ischemic neurons, except for one dog with CA 90 mins at 10°C that showed also edema in the thalamus and dentate nucleus, and another dog of the same group that had also an infarcted area in the dentate nucleus.

Cognitive function testing in the one dog with OPC 1 after CA 60 mins at Tty 20°C, and in the one dog with OPC 1 after CA 90 mins at 10°C, demonstrated that both were able to meet the criteria for successfully learning the cognitive task at 3–6 months after CA. Neither of these two CA dogs performed worse than the normal dog without CA.

Extracerebral Outcome. Extracerebral malfunction was transient, and morphologic changes at 72 hrs were not severe in both studies (Table 2). Variables reflecting gross cardiovascular-pulmonary function were restored to normal during controlled ventilation, except for the four included dogs with complications described previously. At 24 hrs, there were no major increases of alveolar-arterial Po_2 gradients; no dog was hypoxemic. Liver function test values were tran-

siently impaired in all 72-hr survivors; serum glutamyl oxaloacetic transaminase values increased from baseline to 24 hrs and then decreased, but they remained above normal until 72 hrs. Serum γ-glutamyl transpeptidase and bilirubin were within the normal ranges at baseline, 24 hrs, and 72 hrs. Kidney function seemed to be preserved; urine flow ceased during CA and for 1–2 hrs after CA, then at 120 mins after ROSC, achieving normal (baseline) serum creatinine levels at 24 hrs and 72 hrs. Creatinine clearance varied greatly between dogs, independent of group assignment.

Necropsy after CA 60 mins at Tty 20°C revealed moderately hemorrhagic areas in the gastric mucosa in only one dog. In the Tty 15°C group, hemorrhagic consolidation in one lung lobe was found in three dogs, moderate hemorrhagic areas on the liver surface in two dogs, and mild to moderate hemorrhagic areas in the mucosa of the small intestines in two dogs. In the 10°C group, the one dog with fever had lobar pneumonia, and the dog with hemorrhagic diarrhea had mild hemorrhagic areas on the surface of the liver and the rectal mucosa. Necropsy after CA 90 mins at Tty 10°C revealed mild to moderate hemorrhagic areas on the surface of the liver in three of five dogs, hemorrhagic gallbladders in two of five, and mild hemorrhagic areas in the mucosa of the rectum in the one dog with hemorrhagic diarrhea. Necropsy after CA 120 mins at 10°C revealed moderate hemorrhagic areas on the surface of the liver and gallbladder in two of five dogs, mild hemorrhagic areas in the gut mucosa in two of four dogs, and moderate hemorrhagic areas in the gastric mucosa in one dog. None of the 22 necropsies

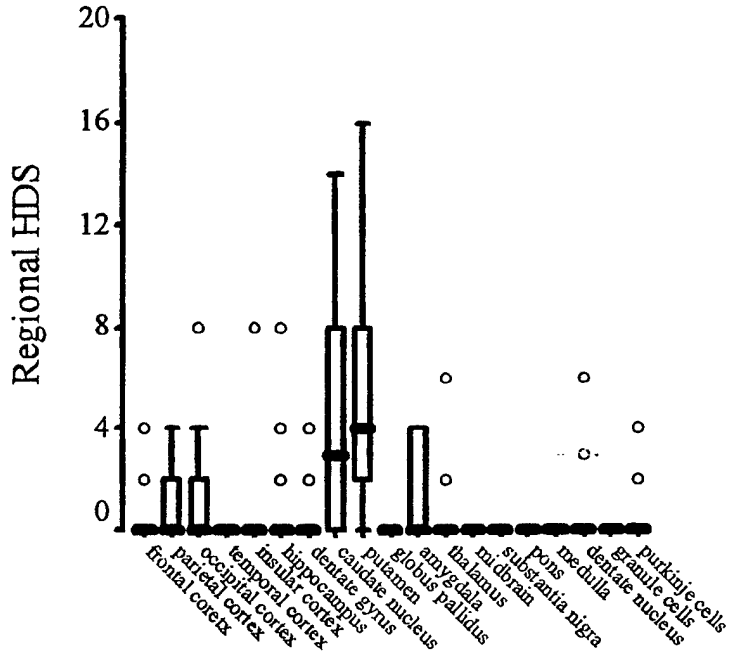

Figure 3. Regional brain histologic damage scores (*HDS*) at 72 hrs after exsanguination cardiac arrest of 60–120 mins. Boxes represent interquartile ranges. The line across each box indicates the median. The whiskers are the highest and lowest values. *Circles* indicate extremes (values more than three box-lengths from the upper or lower edge of the box).

Table 2. Variables for liver and kidney function

	Study A (60 Mins CA)							Study B (Tty 10°C)								
	Tty 20°C (n = 6)			Tty 15°C (n = 5)				Tty 10°C (n = 3)			90 Mins (n = 6)			120 Mins (n = 4)		
Dog	1	2	3	1	2	3	4	1	2	3	1	2	3	1	2	3
Serum GOT, IU/L																
BL	Na	26	46	26	29	23	29	26	26	19	33	22	29	44	41	27
24 hrs	545	545	1717	495	1227	1083	1005	2827	625	227	738	593	246	571	680	1596
72 hrs	230	184	1234	169	350	262	453	509	na	41	217	147	277	392	279	1009
Serum GGTP, IU/L																
BL	Na	9	10	7	6	5	5	6	6	6	8	5	8	5	13	7
24 hrs	6	10	9	11	16	6	7	7	6	5	8	11	8	8	10	8
72 hrs	8	11	11	9	7	9	12	7	na	6	9	8	8	7	11	17
Serum bilirubin, mg/dL																
BL	Na	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.6	0.1
24 hrs	0.2	0.2	0.1	0.4	1.1	0.3	0.1	0.2	0.2	0.2	0.2	0.4	0.1	0.3	0.3	0.2
72 hrs	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.2	na	0.2	0.2	0.2	0.2	0.3	0.3	0.5
Serum creatinine, mg/dL																
BL	Na	0.8	0.7	1.5	0.9	0.8	1.5	0.7	0.6	0.6	0.6	1	0.9	1	0.8	0.7
24 hrs	0.6	0.5	0.6	1.3	0.5	0.8	0.9	0.6	0.5	0.3	0.5	0.8	0.8	0.7	0.8	0.7
72 hrs	0.6	0.6	0.6	1.5	0.4	0.9	0.8	0.8	na	0.8	0.7	0.9	0.9	0.7	0.9	0.8
Creatinine clearance, mL/min/kg																
24 hrs	6.4	5.6	2	1.8	5	5.1	2.7	6.7	6.1	6	7.1	2.8	3.2	4.4	1.5	4.4

CA, cardiac arrest; Tty, tympanic temperature; GOT, glutamyl oxaloacetic transaminase; BL, baseline; GGTP, γ-glutamyl transpeptidase. Normal values in dogs: serum GOT, 10–50 IU/L; serum GGTP, 0–6 IU/L; serum bilirubin, 0.0–0.3 mg/dL; serum creatinine, 0.7–1.5 mg/dL; creatinine clearance, 2.9–4.5 mL/min/kg.

revealed macroscopically necrotic ileum, as is often seen after severe shock states.

DISCUSSION

Suspended animation for delayed resuscitation is being researched systematically to buy time for patients with temporarily unresuscitable CA, to give them a chance to survive without brain damage (4, 5). This clinically relevant exploratory study in dogs is one of a series of systematic studies of exsanguination CA (2–22, 42–44). Trauma surgeons would like a preservation time of ≥60 min for transport and surgical hemostasis. We determined in study A the lowest temperature needed to preserve the organism for 60 mins and in study B the longest possible duration of preservation with Tty 10°C. These studies are the first demonstration of profound hypothermic preservation induced after the onset of no-flow of 60–120 mins, under simulated emergency conditions and with intensive care to 72 hrs. Complete recovery was documented quantitatively in terms of overall performance (OPC) and cerebral function (NDS), gross morphology of all organs, and total brain histologic damage scores (HDS). Brain histologic damage was evaluated by one pathologist who was blinded to hypotheses and group assignments. Interpretation of the results has limitations because the model is difficult and—although clinically relevant—is clinically not fully realistic.

Our results demonstrate the following: First, it is possible to rapidly induce preservative deep and profound hypothermia via a single large-volume cold saline flush into the aorta, starting the flush within the critical 5 mins after the onset of normothermic CA (45), without the need for CPB or heat exchanger. Requirements for use of this approach in the field are still to be worked out. They include rapid access to aorta and vena cava via an automated "smart catheter" approach with chest closed. For hospital emergency room use, it will involve resuscitative thoracotomy and trocar insertion of a balloon catheter into the thoracic aorta and opening the right atrial appendix for flush drainage. All this should be possible within a few minutes. Several liters of fluid will have to be ready in cold storage, and a portable cooling-pumping device is needed. Second, an aortic cold flush to Tty 15°C, starting 2 mins after onset of CA, can preserve viability of the organism, including the brain, for a no-flow time of up to 60 mins; all five dogs achieved OPC 1, whereas Tty 20°C resulted in four of six dogs having hind-leg weakness (OPC 2). Third, an aortic cold flush to Tty 10°C can preserve viability of the organism, including the brain, reproducibly for a no-flow time of up to 90 mins and in some cases for a no-flow time of even 120 mins. Fourth, to achieve preservation during CA 60–120 mins, distribution of the cold flush must include the spinal cord and abdominal viscera (see later). Fifth, for CA 120 mins no-flow, many unknown variables need to be clarified to explain the variable outcomes; theoretically optimized pharmacologic solutions at 2°C should be explored in comparison with saline used so far (42, 43). Sixth, with our model's intensive care unit life support we are encouraged by the fact that the cooled extracerebral organs recovered fully with respect to function after CA 120 mins, perhaps because the endothelium of the microcirculation was protected by cold plus washout of blood before stagnation or thrombosis. Seventh, this nontraumatic model and systemic heparinization for CPB used in this study are clinically relevant for exsanguination from a lacerated large vessel but not for major diffuse tissue trauma, which causes a major inflammatory response and worsened coagulopathy, in addition to that due to hemodilution, hypothermia, ischemia, CPB, and reactive oxygen species (44). Recently, adding trauma to our Tty 10°C model, without systemic heparinization, use of a heparin-bonded CPB circuit, and use of fresh whole blood transfusion, we have achieved intact survival after CA of 60 mins but not yet after longer no-flow (44).

Hypothermia exerts its beneficial effects not merely by reducing oxygen requirement (uptake) (18, 46) but through the synergism of multiple mechanisms, such as preservation of adenosine 5'-triphosphate (47) and reduction of excitotoxicity (48), edema (49), free radical reactions (50), and inflammation (51, 52). Protective-preservative profound or ultraprofound hypothermia during CA 60–180 mins, induced with CPB before the insult, in animals, has been reported previously by us (20–22) and others (23–30). In one study, esophageal temperature was reduced to near 0°C and CA extended to 180 mins; three of 12 dogs survived (30). When in this study esophageal temperature was reduced to only 3°C and life support was optimized, five of seven dogs survived without histologic brain damage (30). Details of histologic evaluation are critical. We have learned since the 1970s (38) that proof of "survival without brain damage" requires histologic search for selectively vulnerable ischemic neurons, in many selectively vulnerable regions, by using systematic, semiquantitative examination and scoring of lesions throughout the entire brain, as in this study (37). Hind-leg weakness can recover to normality after 3–15 days (26, 29). Since in our study all dogs with hind-leg weakness were killed at 72 hrs and the spinal cord did not reveal any histopathology, it is unclear if this deficit would have eventually recovered completely.

Flush to Tty 10°C seems to enable preservation of the organism for up to CA 120 mins but not reliably (Fig. 2); the one dog that achieved only OPC 3 was conscious but not alert and surprisingly had only very mild histologic brain damage. Longer observation might have resulted in functional recovery. In previous studies with shorter CA and mild hypothermia, no improvement of outcome was observed after 72 hrs (34, 37–40). Possible explanations of variable outcomes after CA 120 mins include inhomogeneous, multifocal lack of cold flush perfusion. Future considerations for enhancing preservative hypothermia might include adding a vasodilator to enhance homogeneous fluid distribution, increasing flush pressure, and using a more physiologic flush solution than saline (42, 43, 53–56) and new drugs (15).

The significance of minimal to mild histologic brain damage at 72 hrs, seen in the majority of dogs in our study, is uncertain. We previously established significant correlations between total HDS and NDS at 3–4 days after normothermic ventricular fibrillation CA of 5–20 mins no-flow (6, 16, 17, 34, 37–40). Less clear-cut correlations after longer CA can be explained by extracerebral organ failure (poor OPC) and morphologically normal brain, as in this study, or improved cerebral function in the presence of unmitigated morphologic damage in "silent" brain regions (15). In a previous study by others (27), dogs after CA 105 mins at 5–10°C showed microscopic changes in the brain although cognitive function seemed normal. It is encouraging that in our study, cognitive function tested in two dogs 6 months after CA 60 or 90 mins was the same as in one control dog without CA. These explorations were carried

out during the development of a new method of assessing cognitive function in dogs.

Further development of the potentials of "suspended animation for delayed resuscitation" for presently unresuscitable exsanguination CA (3, 4) must go beyond the pathophysiologic and pharmacologic outcome studies performed so far: First, comparison of outcome with simulated present standard care is not necessary, because we simulated clinical scenarios in which thoracotomy, laparotomy, and fluid infusion have not been clinically effective (3, 4). Second, we are planning clinical feasibility trials in emergency departments of trauma centers for exsanguinating trauma patients arriving pulseless or becoming pulseless under observation. Such patients, typically with penetrating truncal injuries, usually receive emergency thoracotomy (3, 4, 55, 56). Rapid thoracotomy and aortic cannulation under vision, with available catheters (44, 55), could preserve viability during CA until hemostasis is achieved, to be followed by reperfusion and slow rewarming by CPB. The right atrium or vena cava will have to be drained for decompression. The flush and drainage catheters can later serve CPB. Our results in large dogs suggest that in humans, ≥18 L of flush fluid at 2°C would be needed to achieve Tty 15°C. This would preserve the organism for CA of up to 1 hr. A cooling container for storing such a large volume of cold fluid and a pump are needed. Continued asanguinous low-flow by CPB, at profound hypothermia, can preserve the organism for >2 hr (53). The sites of large-vessel injuries will influence where the balloon of the aortic catheter is to be placed, as inserted via the femoral artery or via thoracotomy. Third, ideally, CPB for cooling, reperfusion-rewarming, and prolonged cardiopulmonary support should be available in the emergency departments of major trauma hospitals (4, 17, 57, 58). For emergency departments under austere conditions, however, resuscitation from 10°C without CPB, by using manual heart pumping and intrathoracic warm saline, should be explored. Fourth, for combat casualty care (4, 59), access to the aorta without thoracotomy, perhaps by a still to be developed "smart catheter" and a portable cooling/pumping device, is needed. Fifth, we recently have seen improved prevention beyond that achieved with cold saline flush by using a more physiologic brain preservation solution (54), without or with the antioxidant tempol (42, 43). Sixth, normovolemic CA (i.e., normothermic sudden cardiac death outside hospitals) resists attempts at ROSC by cardiopulmonary resuscitation in 50% of cases (60, 61). It is possible that some of these deaths could be prevented with some still to be determined modification of "suspended animation," to bridge viability until the initiation of prolonged CPB. This and other novel possibilities for the suspended animation concept deserve exploration in animal models.

CONCLUSIONS

We conclude that the rapid induction of profound cerebral hypothermia by aortic flush of cold saline to Tty 10°C, immediately after the start of exsanguination CA—which in most cases cannot be resuscitated with current methods—can achieve survival without functional or histologic brain damage, after CA no-flow of 60 or 90 mins and possibly 120 mins.

We recommend clinical feasibility trials, starting in major trauma hospital emergency departments, of suspended animation for delayed resuscitation in cases of exsanguination CA from penetrating injuries, treated with emergency thoracotomy, and rapid insertion of a flush catheter into the thoracic aorta under vision. More research and development are needed for the suspended animation approach in cases of blunt tissue injuries with coagulopathy and for out of hospital scenarios requiring percutaneous vessel access and a portable cooling-pumping device.

ACKNOWLEDGMENTS

Edwin Klein, VMD, performed histologic evaluation of the dogs' spinal cords. Sherman Culver, Nikolas Dedousis, Jeremy Henchir, Yuichi Sakai, William Stezoski, Jason Stezoski, and Murugan Subramanian helped with ICU life support. Alan Abraham helped with cognitive function tests. Patricia Boyle helped with editing the manuscript.

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Therapeutic Hypothermia for Severe Traumatic Brain Injury

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HYPOTHERMIA HAS BEEN RECOMMENDED IN THE treatment of severe traumatic brain injury (TBI) since at least the 1800s.[1-7] By the mid 1960s, moderate hypothermia (28°C-32°C) had become part of the routine treatment of patients with severe TBI in a number of centers worldwide.[8] However, by the early 1980s, moderate hypothermia for TBI had fallen out of favor because of infectious complications associated with its prolonged and uncontrolled use.[9] In contrast, hypothermia has remained an accepted treatment for refractory intracranial hypertension in both adults and children.[10] In the 1990s, there was renewed interest in the application of mild (33°C-36°C) hypothermia in experimental incomplete cerebral ischemia and cardiac arrest.[11-14] A favorable effect of hypothermia has been reported in more than 90% of the 40 reports published by numerous laboratories using experimental models of TBI.

Since 1992 more than 25 clinical studies have reported effects of therapeutic hypothermia on outcome of TBI, as well as its secondary injury mechanisms and complications after TBI.[15-24] Several of these have been randomized controlled trials (RCTs). Much of the recent clinical work on therapeutic hypothermia in clinical TBI has been performed in Asia and Australia.[17-24]

In this issue of THE JOURNAL, McIntyre and colleagues[25] report a systematic review of 12 trials of therapeutic hypothermia involving 1069 patients. The results demonstrate an overall beneficial effect of moderate or mild hypothermia (32°C -33°C) in severe TBI, with a 19% relative reduction in the risk of death and a 22% relative reduction in the risk of poor neurological outcome compared with normothermia. The data suggest favorable effects for hypothermia durations of 24 or 48 hours, or longer; a target temperature of 32°C to 33°C; and a duration of rewarming of 24 hours or less.

The findings of this systematic review also suggest that some patients may benefit most from a longer duration of cooling, that is, 48 hours or more. It is possible that when used for refractory intracranial hypertension after severe TBI, the optimal use of hypothermia may require titration to effect, rather than application of a single protocol to all patients.[20] In fact, beneficial effects on secondary injury mechanisms may have occurred in patients treated with mild or moderate hypothermia for greater than 48 hours, despite the established risks of complications from prolonged moderate hypothermia.[9,14,18]

Given that slow rewarming has been found to be optimal in laboratory studies,[26] it is surprising that McIntyre et al found that more rapid rewarming, within a 24-hour period of discontinuing hypothermia, conferred greater clinical benefit. However, this finding may not contradict existing laboratory data, because those experiments compared rewarming intervals of minutes vs hours, rather than days. It is likely that patients who tolerate rewarming at a rate of 1°C every 4 hours experience no therapeutic benefit but would have only greater risk of complications with slower rewarming, which could be important because some studies have used rewarming rates as low as 1°C per day.[18]

However, systematic review of these clinical data has many limitations. For example, because the use of hypothermia cannot be blinded, single-center studies may be inherently biased. However, the recent positive results of clinical trials of the use of mild hypothermia after resuscitation from cardiopulmonary arrest in adults[27,28] argues against this possibility.

See also p 2992.

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Also, most patients in recent studies of therapeutic hypothermia in TBI and many of those included in the systematic review of McIntyre et al are from single-center trials. In these studies, cerebral perfusion pressure–targeted treatment was relatively homogeneous, hypothermia was often titrated to optimal depth or duration,[15,17-24] and other aspects of care may have been delivered in a consistent fashion within that center. These factors may limit the generalizability of these findings.

The findings of McIntyre et al contrast with those of a multicenter RCT by Clifton et al,[16] which failed to demonstrate a beneficial effect of hypothermia on outcome after severe TBI in adults. However, several important limitations in that trial were later identified.[29] For instance, although a cerebral perfusion pressure–targeted therapeutic protocol was used in the study, the means by which that therapeutic goal was achieved varied between centers and may have created an impossible challenge for therapeutic hypothermia, despite its consistent benefit in experimental models of TBI. Furthermore, the trial by Clifton et al had a long delay (mean 8.4 hours) in achieving target temperature using surface cooling and gastric lavage. However, the delay in reaching target temperature is substantial in most published clinical trials of hypothermia, even those included in the review of McIntyre et al.

The positive results in clinical trials of mild hypothermia in cardiopulmonary arrest have contributed to renewed interest in the application of hypothermia across a variety of applications, including severe TBI. First, Bernard et al[30] reported that cooling can be rapidly initiated by the intravenous administration of 30 mL/kg of iced (4°C) crystalloid solution in patients who survived cardiac arrest and are comatose. This approach reduced core temperature by about 2°C over 30 minutes and was well tolerated even after cardiopulmonary arrest and resuscitation. Compelling data from other experimental models of cardiopulmonary arrest and TBI suggest that rapid cooling maximizes the benefits of mild or moderate hypothermia. This use of cold intravenous fluids may represent a logical strategy for future clinical trials for use of hypothermia in severe TBI. Sustaining the initial reduction could be achieved with either an intravenous catheter,[31] veno-venous blood cooling,[32] or possibly surface cooling.

Second, studies in experimental TBI have suggested that moderate levels of hypothermia are needed to control intracranial hypertension.[13,14] However, recent clinical studies indicate that treatment with mild hypothermia is often successful at controlling intracranial pressure, even in many cases refractory to medical management. Tokutomi et al[22] evaluated the effect of temperature level on intracranial pressure during cooling to 33°C in 31 adults with severe TBI and found that the decrease in intracranial pressure and improvement in cerebral cranial pressure was greatest at 35.5°C. Such a moderate level of titrated cooling, if effective, might reduce the risk of adverse effects. Further clinical investigation and application of titrated mild hypothermia in patients with severe TBI are needed.

Third, recent animal studies by Statler et al[33] suggest that creating a state of poikilothermia is essential to prevent deleterious consequences of stress during induction and maintenance of moderate hypothermia after experimental TBI. Sedatives, narcotics, and neuromuscular blockade are generally recommended. Preliminary work in an animal model of cardiopulmonary arrest[34] suggests that novel pharmacological agents may facilitate rapid cooling while minimizing the stress response. Optimal pharmacological adjuncts in mild and moderate hypothermia represent an important future area of research and clinical application. Hypothermia appears to have extremely powerful effects on some but not all secondary injury mechanisms,[35] and the combination of hypothermia and pharmacological therapies to prevent oxidative stress may be particularly promising.[36,37]

Fourth, following severe TBI, patients with secondary insults, such as hypotension or hypoxemia, have consistently poor outcomes[38] but are routinely excluded from clinical trials, including those of hypothermia treatment.[15,16] It will be important to examine mild or moderate hypothermia in this clinical setting, where the contribution of ischemic mechanisms of secondary damage would suggest added value of mild cooling after resuscitation.

Fifth, both the duration of the application of hypothermia necessary for optimal effect and the rate of rewarming remain unclear, and may not be the same for all cases. Recent work by Iida et al[24] raises the possibility of using the occurrence of hyperemia (detected by transcranial Doppler) as an early predictor of the development of intracranial hypertension during rewarming of patients with severe TBI. This preliminary report reinforces the important concept of using physiological or biochemical parameters, rather than using a single fixed regimen for all cases, to determine the optimal duration of hypothermia or rate of rewarming. It is not yet clear what the optimal parameters are, but the concept merits further study.

Finally, considerable laboratory evidence suggests that hyperthermia is deleterious after severe TBI, and thus should be avoided. Catheter-based cooling for continuous temperature control,[31,32] has been found to be feasible in patients with a number of diagnoses in a neurointensive care unit.[31]

Several clinical trials in patients with severe TBI are now ongoing, including 2 pediatric trials and a new adult trial, as described by McIntyre et al.[25] Because surface cooling is generally slow and unreliable, future clinical investigation on the use of hypothermia in patients with severe TBI should consider the following: rapid induction of cooling via intravenous administration of cold crystalloid; rigorous maintenance of temperature control with intravascular cooling devices; initially targeting mild levels of hypothermia; subsequent titration of the level and duration of hypothermia to clinical, physiological, and biochemical effect; and thor-

ough characterization of the consequences of various rewarming paradigms. Although mild or moderate hypothermia is an accepted therapy for refractory intracranial hypertension after TBI in both adults and children, whether it should be used as a first tier therapy and exactly how it compares with other second tier therapies are 2 key questions that remain to be determined in clinical trials. Additional investigation of hypothermia in experimental and clinical brain injury should define the mechanisms underlying its beneficial, and potential deleterious effects, and translate that knowledge into optimized combinations of hypothermia and novel pharmacological strategies.

Funding/Support: The authors are supported by grants NS 30318 and NS 38087 (Dr Kochanek) from the National Institutes of Health and DAMD 17-01-2-0038 (Dr Safar) from the US Army.

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For: *A Textbook of NeuroIntensive Care*. Layon AJ, Gabrielli A, Friedman WA, editors.
Published by WB Saunders Company
In press

CEREBRAL RESUSCITATION FROM CARDIAC ARREST

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Sections of this chapter are based on ref. 1 and ref. 2 (Safar: Resuscitation of the Ischemic Brain, chapter 17 in *Textbook of Neuroanesthesia* [M.A. Albin, ed], McGraw-Hill, New York, 1997.)

Brain Resuscitation After Cardiac Arrest

Outline

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ABBREVIATIONS

ACD-CPR–	active compression-decompression CPR
AHA –	American Heart Association
ALS –	advanced life support
ATP –	adenosine triphosphate
BLS –	basic life support
BRCT –	Brain Resuscitation Clinical Trial
CA –	cardiac arrest
CBF –	cerebral blood flow
CCM –	critical care medicine
$CMRO_2$, G,L –	cerebral metabolic rate for oxygen, glucose, lactate
CPB –	cardiopulmonary bypass
CPCR –	cardiopulmonary-cerebral resuscitation
CPP –	cerebral perfusion pressure (MAP-ICP)
CPR –	cardiopulmonary resuscitation (BLS-ALS-PLS)
CSF –	cerebrospinal fluid
DNA –	deoxyribonucleic acid
ED –	emergency department
EEG –	electroencephalogram
EMD –	electromechanical dissociation
EMS –	emergency medical services
FBI –	focal brain ischemia
GBI –	global brain ischemia
Hct –	hematocrit
IAC-CPR –	intermittent abdominal compression CPR
ICP –	intracranial pressure
ICU –	intensive care unit
IPPV –	intermittent positive pressure (controlled) ventilation
MAP –	mean arterial pressure
OR –	operating room
OCCPR –	open-chest CPR
PCD –	programmed cell death (apoptosis)
PCO_2 –	CO_2 tension
PEA –	pulseless electrical activity (= EMD)
PLS –	prolonged life support
PO_2 –	oxygen tension
PVS –	persistent vegetative state
ROSC –	restoration of spontaneous circulation
SA –	suspended animation
SCD –	sudden cardiac death
SECPR –	standard external CPR
T –	temperatures: "core T" as rectal (Tr), esophageal (Tes), central venous (Tcv), urinary bladder (Tu), pulmonary artery (Tpa). "Brain T" as tympanic membrane (Tty), nasopharyngeal (Tnp), brain tissue (Tb), epidural (Tep), or intraventricular (Tiv)
TBI –	traumatic brain injury
VF –	ventricular fibrillation
VT –	ventricular tachycardia
WFSA –	World Federation of Societies of Anaesthesiologists

ABSTRACT (SUMMARY)

Normothermic temporary complete global brain ischemia during cardiac arrest (CA) with no-flow of 5 min or longer (the time at which the brain becomes depleted of glucose and energy), initiates extremely complex chemical cascades that lead, after reperfusion-reoxygenation, to a multifactorial postischemic-anoxic encephalopathy. Thus, cerebral resuscitation calls for a multifaceted strategy. Since the 1970s, we have documented that, under controlled normotension after prolonged CA in dogs (or global brain ischemia in monkeys), cerebral blood flow goes through transient cerebral hyperemia to protracted (inhomogeneous) hypoperfusion, which is mismatched to increasing O_2 uptake. Vasospasm, endothelial swelling, blood sludging and coagulation are suspected mechanisms. Hypertensive reperfusion prevents the no-reflow phenomenon, and correlates with better cerebral outcome in dogs and patients. Moderate prolonged hypertensive hemodilution normalizes cerebral blood flow after CA. Thrombolysis looks promising for brain and heart. After 20 years of studies on mechanisms and drugs, by numerous neuroscientists, using cell-culture, brain-slice, and rodent models, vast knowledge was gained about the molecular-cellular role-players. Uni-mechanistic drug treatments proved disappointing, in contrast to hypothermic strategies. Many drugs reported to mitigate the loss of neurons in the hippocampus of rats after incomplete forebrain ischemia (which is clinically unrealistic), when tested for use after CA in dog outcome models (which are clinically realistic) neither improved cerebral function nor reduced histologic brain damage. Thiopental loading after global brain ischemia looked effective in some animal studies, but other animal studies and the first randomized clinical outcome study of cardiopulmonary cerebral resuscitation (CPCR), the Brain Resuscitation Clinical Trial (BRCT), found no significant overall outcome benefit. Similar disappointing results were seen in patients with calcium entry blocker therapies. Titrated

escalating high doses of epinephrine in patients improved restoration of spontaneous circulation rates, but not good cerebral outcome rates. Randomized clinical outcome trials are not controllable and therefore should not be the "gold standard" for setting clinical guidelines for CPCR. More reliable documentation comes from controlled models in large animals with prolonged CA and clinically realistic intensive care life support to outcome evaluation.

Protective-preservative *moderate* hypothermia *during* elective surgery with CA has been in use since the 1950s. *Resuscitative hypothermia* after normothermic CA, tried around 1960 and then given up for 25 years, was revived in the mid-1980s with the discovery of beneficial effects of *mild* hypothermia (33-36°C). When combined with hypertension and normocapnia in dogs, mild hypothermia after normothermic CA increases the longest reversible no-flow time from 5 to 10 min. There are now positive outcome results of clinical trials of mild hypothermia after CA in Japan, Australia, and Europe. Resuscitation from prolonged CA may require cardiopulmonary bypass. For presently unresuscitable CA (e.g., traumatic exsanguination; CPR-resistant normovolemic CA), "suspended animation for delayed resuscitation," to preserve brain and organism during CA, has been under laboratory investigation in Pittsburgh for over one decade. In dogs, aortic flush induction of pharmacologic preservation potentials gave disappointing results (with the exception of the antioxidant tempol). Cold flush (2°C) into the aorta, within 5 min of CA, of saline or novel cerebral preservation solutions, can reduce cerebral temperature by 3°C/min and preserve viability of brain and organism during up to 90 min (perhaps even 120 min) no-flow at tympanic temperature of 10°C.

Strategies for new possibilities of increasingly effective cerebral resuscitation from CA in the 21st century include: 1) Reducing normothermic no-flow times to <5 min with life-supporting first aid by lay bystanders (including earliest automatic external defibrillation). 2)

Combining hypothermic and pharmacologic strategies after longer no-flow. 3) Combating the prolonged post-resuscitation disease in all vital organs.

I. INTRODUCTION

A. Definitions (Figure 1)

Cerebral *protection* (pretreatment) and *preservation* (intra-insult treatment) before and during (anticipated) cerebral ischemia are important in the management of patients undergoing elective cerebral or cardiac anesthesia and surgery. Cerebral *resuscitation* is treatment to reverse the insult and support recovery.[1,2] This chapter concerns cerebral *resuscitation* from the temporary, complete global brain ischemia (GBI) of *cardiac arrest* (CA). This topic is relevant for emergency medical services (EMS),[3-5] which should have matured by 2000 C.E. to deliver not merely cardiopulmonary resuscitation (CPR),[7] but rather cardiopulmonary-*cerebral* resuscitation (CPCR).[1,2,6] CPCR leads to intensive care with focus on brain and heart. In many patients who, after a variety of cerebral insults, are brought to the intensive care unit (ICU), either from outside or inside the hospital, the fate of the brain has been determined earlier, during and immediately after the insult. Nevertheless, intensivists should know about novel potentials for cerebral resuscitation, since they are often consulted on such cases outside the ICU, and since brain-focused prolonged life support can mitigate brain damage.

Temporary hypotension with mean arterial pressure (MAP) of about 30-60 mm Hg, can be tolerated by the normal brain, but even mild hypotension can cause permanent brain damage when it occurs in a state of severe hypoxemia, after brain trauma, or in the presence of atherosclerotic cerebral arteries that fail to go into autoregulatory vasodilation. Much of what applies to cerebral resuscitation from the GBI of CA is also relevant for special operations on the brain which require temporary circulatory arrest.

One must differentiate between the temporary, complete GBI of CA (Figure 1); the permanent, complete GBI of panorganic death without resuscitation; the temporary, incomplete GBI of shock states; the temporary or permanent focal brain ischemia of stroke (e.g., cerebral embolism); traumatic brain injury with unifocal, multifocal, or global ischemic components; and a variety of toxic, inflammatory, or degenerative cerebral insults. A treatment that is effective for one of the above conditions may not be effective for another; one effective for protection-preservation during an insult may not be effective for resuscitation; and one effective during incomplete ischemia may not be effective after complete ischemia.

One must further differentiate between ischemic tissue hypoxia or anoxia that is caused by reduced cerebral blood flow (CBF); hypoxic hypoxia that is low arterial PO_2 (PaO_2); and anemic hypoxia that is caused by very low hemoglobin levels (low hematocrit [Hct] or carbon monoxide [CO] poisoning). One must also differentiate between *process* variables during and early after the insult, such as electroencephalographic activity (EEG), CBF, or cerebral metabolic rates for oxygen, glucose, or lactate ($CMRO_2$, CMRG, CMRL); and the much more important *outcome* in terms of cerebral functional and morphologic changes, after maturing of the post-ischemic encephalopathy over at least three days, perhaps weeks.

In ischemic insults like CA (e.g., sudden cardiac death [SCD]), one must further differentiate between the many different mechanisms leading to CA, such as asphyxia, exsanguination, ventricular fibrillation (VF), electromechanical dissociation (EMD) (=pulseless electrical activity [PEA]) with mechanical asystole, or electric asystole; and slow, secondary CA (Figure 1). One must determine or at least estimate *arrest time* (no-flow), *CPR time* (low-flow), and temperature – the most important variables determining cerebral outcome.

Reversal of CA calls for CPCR in three phases, i.e., basic, advanced, and prolonged life support (BLS-ALS-PLS).[1,2] When initiated outside the hospital, the steps of resuscitation are to be delivered throughout the EMS system, i.e., from scene via transportation to the most-appropriate hospital's emergency department (ED), operating room (OR), and ICU.[3-5] CPCR and EMS[3] combined are now called the "chain of survival,"[4,5] which is only as effective as the weakest step of CPCR in the weakest link of EMS.

When talking about *hypothermia* -- the presently most effective cerebral preservation and resuscitation strategy -- one must differentiate between controlled (therapeutic) and uncontrolled (accidental) hypothermia; between temperature levels, such as normothermia (37-38°C), and mild (33-36°C), moderate (28-32°C), deep (16-27°C), profound (5-15°C), or ultraprofound (< 5°C) hypothermia; and between different temperature-monitoring sites. Temperatures are measured for the brain as brain tissue (Tb), intraventricular (Tv), epidural (Tep), tympanic membrane (Tty), or nasopharyngeal (Tnp) temperature; and for the body-core as esophageal (Tes), central venous (Tcv), pulmonary artery (Tpa), rectal (Tr), or urinary bladder temperature (Tu).

B. Importance

The *socio-economic* importance of attempts at mitigating any type of cerebral insult is obvious because survival in persistent vegetative state (PVS) is an enormous burden to families and society; and conscious survival with partial paralysis, aphasia, mental and cognitive disturbances or other neuro-psychologic deficits, is not only a burden to family and friends, but also torture for patients themselves. Billions of dollars are spent each year on the care of such patients. The *clinical* importance of resuscitation from GBI goes beyond the cases of CA, since

all resuscitation and intensive care life support efforts for the organism should focus on the brain. The *scientific* importance of research into cerebral resuscitation from CA is considerable because CA is experimentally controllable and gives clues for the treatment potentials also for the more variable and more complex insults like stroke, TBI, or shock.

Recent attempts at implementing national CPR guidelines[7] or international CPCR guidelines[1] through community-wide EMS systems[3] have yielded suboptimal results.[2,7-21] At present, among pre-hospital or in-hospital CPR attempts outside special care units, fewer than 50% have achieved restoration of spontaneous circulation (ROSC), and fewer than 10% overall have resulted in conscious survival. About 10-30% of long-term survivors of CA have some permanent brain damage. The main problems seem to be pre-existing heart disease obviating ROSC, and long arrest (no-flow) times due to delayed, suboptimal resuscitation, i.e., "too little too late." Remedies proposed include life supporting first aid (LSFA) skill acquisition by all fit humans on earth, starting in grammar school,[22] for the immediate initiation of resuscitation, and uniform reporting for community-wide ongoing evaluation of outcome.[15-24]

In 1961, Safar assembled the CPCR system,[1,25,26] which has consisted of 3 phases – basic, advanced, and prolonged life support (BLS-ALS-PLS) -- with 3 steps each: step A (airway control),[27-30] step B (breathing control),[27,31,32] and step C (circulation support)[33-35] are phase I, BLS.[36,37] Cerebral resuscitation starts with optimizing BLS.[1] To rapidly achieve ROSC, BLS has been followed by steps D, E, and F (drugs and fluids, electrocardiography, and fibrillation treatment) which are phase II, ALS, to rapidly achieve stable, optimal O_2 delivery.[38-58] Now, BLS and ALS deserve some modifications (see later). Steps G (gauged), H (humanized = brain-oriented, with hypothermia [?]), and I (intensive care) are phase III, brain-oriented PLS.[59-63] For PLS, respiratory ICUs were pioneered in Scandinavia in the early 1950s, and physician-staffed

medical/surgical ICUs for any vital organ failure began in 1958.[59] Critical care medicine (CCM) rapidly spread around the world.[60-63] Ideally, for in-hospital CA, it is feasible for BLS-ALS to achieve ROSC within the critical 4 to 5 min. Around 1970, research began into *cerebral* resuscitation from CA, and CPR was extended to CPCR.[1,2,25] Term and concept of CPCR were adopted by international guidelines in the 1980s,[1] but the American Heart Association (AHA) guidelines of 2000 C.E. have not yet given appropriate priority to cerebral resuscitation potentials (Figure 2).[7,9,10]

Promptly initiated, vigorously performed BLS (low flow) can often sustain the viability of heart and brain even during prolonged transport.[64-67] Reducing the response time of mobile ICU ambulances to less than 10 minutes is usually not feasible.[7,8] The currently quoted maximal period of normothermic no-flow, induced suddenly, as by VF, that is consistently reversible to complete recovery of cerebral function and structure, is 4 to 5 minutes,[17-21,68-77] shorter for CA caused by asphyxiation.[78] If the brain-tolerated no-flow time were extended to 10 minutes, mobile ICU ambulances could arrive in time for an estimated 100,000 additional lives to be saved with good neurologic outcome in the United States every year. Recent dog outcome studies have shown that this might be possible (see later).[2] A single pharmacologic, penicillin-like "magic bullet" with a unifactorial breakthrough effect may never be found, because the postischemic-anoxic encephalopathy, i.e., the cerebral postresuscitation disease, is complex and multifactorial (Figures 3 and 4). Because of this complexity, one of us (PS) has called for the design and evaluation of treatments that attack several deleterious mechanisms simultaneously.[2,71-75] Mild resuscitative hypothermia proved to be just that (Figure 5).[2] The CPCR alphabet of 1961[26] and present AHA guidelines[7] therefore must be updated (Figure 6).

Optimism is justified,[79,80] since most (but not all) cerebral neurons,[81-86] and cardiac myocytes,[87-90] can tolerate much longer periods of complete normothermic ischemic anoxia in vivo than previously assumed. A few selectively vulnerable dead cerebral neurons can impair human mentation, whereas the heart can pump in spite of up to 40% of myocytes lost. Normothermic no-flow of 5-20 minutes, in animals and patients, can be reversed to cardiovascular survival[74] and recovery of cerebral oxygen metabolism,[76,84] but not survival of all cerebral neurons.[74,83] A clinically realistic combination of CBF promotion and mild hypothermia, however, has recently achieved complete functional recovery in dogs after 11 minutes of normothermic CA (no-flow) (Figure 5)[2] (see later).

II. HISTORY

A. Cardiopulmonary Resuscitation

Occasional attempts to reverse sudden coma, airway obstruction, and cessation of breathing have been made since prehistoric times;[91,92] however, potentially reversible apnea and pulselessness (CA) was not recognized until the Renaissance.[93] The *early history* of CPR[91] has many sparks, which failed to benefit patients over centuries probably because of lack of communication and collaboration among laboratory researchers, clinicians, and rescuers. Searching for effective resuscitation measures was provoked by the introduction of general anesthesia, which, starting in the late 1800s occasionally led to airway obstruction, apnea, or pulselessness, as the introduction of asepsis enabled laparotomies which required deep anesthesia. Modern effective CPR (without thoracotomy), however, did not come about until the 1950s.[25-42]

The *recent history* of modern standard external CPR (SECPR)[92] shows a series of landmark developments, starting in the 1950s: Proof (in curarized adult human volunteers without tracheal tube) that soft-tissue obstruction of the upper airway in unconscious patients can be prevented or corrected by backward tilt of the head, forward displacement of the mandible, and opening of the mouth (step A).[27,28] Proof that ventilation with the operator's exhaled air is physiologically sound;[27,31] and that mouth-to-mouth (nose) ventilation is superior to manual chest-pressure arm-lift methods in adults (step B).[27,31,32] The serendipitous rediscovery, laboratory documentation, and first clinical uses of emergency artificial circulation by external cardiac (chest) compressions (step C);[33,34] combining steps A, B and C into BLS;[36,37] the first successful electric defibrillation of a human heart via thoracotomy;[40] and the first successful external electric defibrillation and pacing of human hearts.[42] Finally, the concepts of "hearts too

good to die" (reversible sudden cardiac death) (Beck, 1960) and "brains too good to die" (Safar, 1970).

B. Cerebral Resuscitation

The extension of *CPR to CPCR* occurred conceptually in 1961.[25,26] Guthrie of Pittsburgh drew attention to the brain in resuscitation research much earlier.[94] It required experimental proof, which began around 1970: 1) The recognition by Hossmann that many cerebral neurons can tolerate longer no-flow times than previously assumed;[81-84] 2) the description by Negovsky and his associates of the cerebral post-resuscitation disease;[70,95-99] 3) the development by Safar of clinically realistic GBI and CA models in animals high on the phylogenetic scale (monkeys, dogs), including intensive care life support over several days to let the encephalopathy mature, and to evaluate outcome,[2,74,77,100-103] and the design by the Pittsburgh group of the first (multicenter) randomized clinical trials of CPCR, the Brain Resuscitation Clinical Trials I-III (BRCT). Subsequently, many neuroscientists have added knowledge about the mechanisms of cell death. This has revealed the increasingly complex molecular and cellular mechanisms of postischemic encephalopathies (Figure).[2,76,133] Many seemingly positive trials of novel drug treatments in rat models of incomplete forebrain ischemia[105] which are clinically not realistic, could not be duplicated in outcome models in dogs.[2,80] Rodent models of CA[106-116] are clinically more relevant, but long-term life support is difficult or impossible. There are many models of hypoxic insults to neurons which are clinically not realistic, ranging from in vitro models[117] to increased intracranial pressure (ICP) in dogs.[118]

For almost 100 years before 1970, some pathologists, neurosurgeons, neurologists and -- since the 1950s, neuroanesthesiologists -- studied the brain after operative trauma, accidental

trauma, intracranial hemorrhage, or focal ischemia (stroke). Present cerebral resuscitation researchers stand on the shoulders of the pioneers of therapeutic cerebral hypothermia of the 1950s and 1960s. In the 1960s, neuropathologists documented that after GBI there is delayed dying of scattered cerebral neurons. Studies of postischemic encephalopathies were encouraged by the introduction of CBF methods.[119-128] Concerning traumatic shock (i.e., incomplete GBI), which has been studied for extracerebral organ failure since the 1930s, the brain has remained relatively unexplored. Around 1970, Hossmann[81-84] showed that the majority of cerebral neurons (by far not all) in cats or monkeys can tolerate up to 60 min of complete normothermic GBI -- in terms of recovery of EEG activity and protein synthesis -- provided reperfusion is good. The Pittsburgh group, after an EEG recovery study in 1968,[129] documented in 1971, for the first time, the transient hyperemia-protracted cerebral hypoperfusion sequence after prolonged CA in dogs (Figure 3).[123,124] This hypoperfusion (Figure 3) had to be overcome to improve outcome.[130-132] How to prevent delayed post-CA dying of selectively vulnerable neurons remains an important challenge for resuscitation researchers.[79] That challenge was partially met through the discovery of *mild* resuscitative (post-CA) hypothermia in the 1980s. For the history of therapeutic hypothermia, see "hypothermic strategies."

The main "incremental risk" of increasingly effective CPCR methods is survival with severe brain damage. Since the mid-1960s, the topics of determination and certification of brain death, and the decision to "let die" regarding patients in prolonged vegetative state after CA, have assumed increasing socioeconomic importance.

III. PATHOPHYSIOLOGY

A. Post-ischemic Encephalopathy

The temporary complete GBI of CA can occur instantly, as in VF; over minutes, as in asphyxiation or exsanguination; or over hours, as in shock or hypoxemia (Figure 1).[1,2] Sudden cardiac death and resuscitation create a cerebral insult that is often caused by the initial no-flow, followed by the incomplete ischemia of CPR (low-flow), and, after ROSC, by the postresuscitation disease in brain and other vital organs.[70,96] While extracerebral derangements after normothermic CA of 5-20 min, ROSC, and controlled normotension seem to be reversible under adequate life support, selectively vulnerable cerebral neurons continue to die. This was first described by Spielmeyer. Main mechanisms, we have learned recently, are post-CA hypoperfusion (Figure 3) and complex destructive chemical cascades (Figure 4).

In normal brain, autoregulation maintains global CBF of about 50 ml/100 g brain per minute, despite cerebral perfusion pressure (CPP) changing between 50 and 150 mm Hg. CPP is mean arterial pressure (MAP) minus ICP or internal jugular vein pressure, whichever is higher. When CPP decreases below about 50 mm Hg, CBF decreases. During incomplete ischemia (e.g., shock or CA with external CPR), the viability of normal neurons seems to be threatened only when CPP decreases to or below 30 mm Hg,[134-138] CBF to less than 15 ml/100 g of brain per minute,[137] or cerebral venous PO_2 to less than 20-25 mm Hg[137-140] (normal value > 40 mm Hg). The brain apparently tolerates low-flow (e.g., global CBF 10% of normal, i.e., 5 ml/100 g of brain per minute) better than no flow;[141] however, trickle-flow (CBF less than 10% normal) can be worse than no-flow.[142]

With sudden circulatory arrest at normothermia, loss of brain O_2 stores[143] and unconsciousness (in normal humans with neck tourniquet inflation)[144] occur within 10 to 20

seconds. For complete reversibility, the 4-5 minute limit established clinically[68-78] is supported by evidence that brain glucose and ATP stores are depleted[145,146] and the cell membrane ion pumps arrested[143,147,148] within 3 to 5 minutes of normothermic circulatory arrest. Distinct events during ischemia (energy loss) and after reperfusion, described in detail in the legend of Figure 4, lead to abnormal activation of lipases, proteases, and nucleases,[2] and suppression of translation, signal transduction, and growth factors.

In dog outcome experiments, VF no-flow of 1-4 minutes is reversed to complete functional recovery and normal brain histology, whereas VF no-flow of 5 minutes[77] or asphyxial asystole of only 2 minutes[78,149,150] is followed by complete functional recovery, but with mild histologic damage in vulnerable regions. The 4-5 minute limit is being challenged by the occasional survival without severe neurologic deficit after normothermic no-flow of 10 to 20 minutes in dogs[74,77,151] or in patients.[86,152,153] These cases might be explained by unrecognized spontaneous mild hypothermia. Normothermic no-flow of 60 minutes was survived in one cat with only slightly abnormal behavior, but with some histologic brain damage.[83] Undoubtedly, multiple factors -- some understood (e.g., spontaneous hypothermia, see below) and others as yet unknown – might explain these occasional "miraculous" recoveries.

During complete cerebral ischemia, calcium shifts,[76,143,154,155] brain tissue lactic acidosis,[156] and increases in the brain of free fatty acids,[157] osmolality,[158] and extracellular concentration of excitatory amino acids (particularly glutamate and aspartate),[159-162] set the stage for reoxygenation injury (Figure 4). Greater cerebral lactic acidosis with incomplete ischemia[156] or prearrest hyperglycemia[163] is followed by greater histologic brain damage. Brain acidosis caused by high CO_2 without hypoxia, however, seems to be better tolerated, at least by the normal brain.[164,165]

Why do some neurons die while neighboring ones survive? Are the more vulnerable neurons those that are more stimulated post-CA (excitotoxicity), or those that had been programmed to die sooner than others without CA, but were triggered by CA into earlier programmed DNA damage? Present research interests include how selectively vulnerable neurons die alongside surviving neurons, predominantly in the CA-1 region of the hippocampus, neocortex, thalamus, and cerebellum (Purkinje cells). There is a possibility that temporary, complete ischemia can damage DNA and thereby trigger programmed cell death of some (scattered) and not other neurons, i.e., "apoptosis" (in Greek, "falling apart").[166] This might differ from primary necrotizing processes and membrane breakdown in the majority of neurons.

Post-insult intracranial pressure (ICP) increases, due to vasogenic cerebral edema, hyperemia (increased blood volume) or cerebral venous obstruction, is more an issue for resuscitation from TBI or severe ischemic stroke or cerebral hemorrhage, than for CA,[123] except for cases of very prolonged CA which is followed by ICP rise to brain death. For ICP control after insults other than CA (or GBI), see the section on hypothermia.

B. Post-ischemic Hypoperfusion (Figure 3)

Cerebral perfusion changes (i.e., CBF changes during normotensive reperfusion after 10 min or longer of normothermic CA no-flow) seem to progress through four stages:

1) Multifocal (heterogeneous) no-reflow, observed immediately with reperfusion.[70,167-172] It is most likely caused by blood cell sludging. It seems to be readily prevented or reversed in animals with normotensive or hypertensive reperfusion[173-186] with or without hemodilution.[130-132,181] These measures improve outcome,[130-132] perhaps even leukocyte adhesion, endothelial swelling and clotting.[172]

2) Transient global "reactive" hyperemia (vasoparalysis), which lasts 15 to 30 min;[123-125,173-180] may be beneficial.

3) Delayed, prolonged global and multifocal hypoperfusion,[123-125,173-180] from about 1-2 h to 12 h after CA and ROSC. Global CBF is reduced to about 50% of baseline, while global $CMRO_2$ which had become zero during complete ischemia, is recovering with reperfusion to moderately low levels in the first 1-2 hours.[187] From 2 hours on, however, $CMRO_2$ returns to or above baseline values, causing a potential mismatching of O_2 delivery vs O_2 demand.[82,123-125,173-175,178] Cerebral-venous PO_2 may decrease to less than the critical level of about 25 mm Hg or $ScvO_2$ to < 50% or cerebral O_2 extraction ratio ($Ca\text{-}vO_2/CaO_2$) to > 50%.[177-179] Hypoperfusion is worse after long arrest times.[188] The pathogenesis of this third stage (protracted hypoperfusion) needs clarification. Vasospasm,[175,189-191] endothelial and tissue edema,[123] blood cell aggregates,[172] leukocyte adhesion and perhaps even intravascular coagulation[192-197] are possibilities. Inflammation, so important after brain trauma,[198] seems less important after GBI,[199-201] although there is a mild response of glial elements.[202] The delayed protracted global cerebral hypoperfusion after CA has been documented also in patients.[203,204]

4) Resolution, when (many hours after ROSC), in dogs, either normal global CBF and $CMRO_2$[174] accompany return of consciousness,[132] or both remain low in comatose patients.[203-205] Most patients going into permanent coma (persistent vegetative state) do not develop intracranial hypertension. After very long normothermic CA, and ROSC, with persistent deep coma, there is first transiently some improvement (narrowing of pupils) only to be followed by fixed, dilated pupils, intracranial hypertension (cerebral edema),[206,207] decrease in oxygen uptake, high cerebral venous PO_2 due to arterio-venous shunting, EEG silence, non-filling of intracranial vessels on angiogram, and the clinical picture of brain death.[1,2,208-213] There is a need to clarify the

pathophysiology of brain death development, in dogs or monkeys, after very long CA and ROSC.

C. Deleterious Chemical Cascades (Figure 4)

What is the *complex multifactorial pathogenesis* of the post-CA encephalopathy? Starting at about 24 hours after arrest, irreversible morphologic changes can be seen by light microscopy in some neurons scattered throughout the brain.[77,103,214-221] Reperfusion leads to scattered neurons in selectively vulnerable regions developing irreversible changes. These start, depending on the insult, at less than 1 hour with vacuolization of mitochondria, seen by electron microscopy. Then follow, in 2-4 days, shrinkage and eosinophilia of cytoplasm on light microscopy (with HE staining) and pyknosis of nuclei, i.e., "irreversible" ischemic neuronal changes. After more than 7 days, absorption of dead neurons leads to a reduction in the number of morphologically normal neurons.[2,69,77,84,101-105,109,110,214-221]

Without reperfusion, all cells of the brain seem to die uniformly as part of panorganic death (Figure 1). Necrosis is the ultimate result of no energy.[219,220] *With* reperfusion, energy is rapidly restored. Why do only some neurons die and why do we see patterns of apoptosis and/or necrosis? The final answer is still elusive. Since the 1970s, many scientists have helped increase our knowledge about how neurons die after temporary GBI in the absence of post-CA secondary ischemia. Derangements *during* GBI, summarized above, set the stage for reoxygenation injury – the cerebral postresuscitation disease (Figure 4, see legend).[2,70-72] *After* normothermic no-flow of 5-20 min duration followed by reperfusion, many secondary derangements can be considered. Brain energy charge, ion pump, and normal pH are restored fairly quickly.[76,83,143] Postarrest inflammatory processes, which are proven to occur after TBI or in ischemic stroke, seem to be

absent or minimal after CA,[198] unless trauma and ischemia are combined.[216] A new area of brain ischemia research is "proteomics," the changes in brain protein concentrations and the recovery of protein synthesis.[222] Even when, after CA of up to 15 min, ICP remains normal,[103,123,124] extracellular-to-intracellular fluid and electrolyte shifts occur early during ischemia (cytotoxic edema).[123,148,206] Vasogenic edema may develop late after reperfusion from very prolonged ischemia.[103,207]

Reoxygenation , although essential and effective in restoring energy charge, can provoke chemical cascades that result in lipid peroxidation of membranes.[223-228] These processes, possibly first triggered by calcium shifts,[229-231] also involve free iron, free radicals, acidosis, catecholamine release,[232] excitatory amino acid release,[159-162] and induction of proteases,[233,234] leading to destruction of membranes and nuclear DNA. Earlier studies suggested no direct nuclear DNA damage after CA.[235,236.] Recent evidence suggests that profound cell death may occur in selectively vulnerable neurons with DNA fragmentation.[237-242] Cerebral viability also depends on the DNA in mitochondria which can be damaged by late calcium fluxes through mitochondrial transition pores (MTP);[241] and mitochondrial cytochrome-c inducing apoptosis. Many of the above cascades (Figure 4) have been partially documented in vitro and in extracerebral organs, but have not been documented convincingly in vivo in the brain. The optimal PO_2 and rate of reoxygenation during resuscitation need to be determined. The increase of lactic acid[156] and excitatory amino acids[159-162] that occur during ischemia are rapidly washed out with reperfusion, and ionic balance is partially restored.[143] Although there might be a delayed postarrest increase in total brain calcium,[230] treatable surges in brain intracellular calcium or glutamate release after arrest remain to be documented. Some of these molecular

changes could be merely epiphenomena of permanent tissue damage, whereas others might explain why dying neurons and dying cardiac myocytes can be found alongside surviving cells.

Extracerebral derangements can worsen cerebral outcome.[70,243] CA in patients with previously sick hearts often is followed by recurrent VF or cardiovascular-pulmonary failure.[15-21] CA in previously healthy dogs is followed by delayed reduction in cardiac output despite controlled normotension.[89] Postarrest pulmonary edema, however, can be prevented by prolonged controlled ventilation.[89,101,102] Intoxication of the brain from post-ischemic viscera has been suggested,[70] but convincing documentation is lacking. After no-flow of 10 minutes in healthy dogs and modern post-CA life support, neither pulmonary failure nor permanent renal or hepatic dysfunction appear to occur.[89,244,245] Disseminated intravascular coagulation (DIC) is a possibility.[197] Blood derangements include aggregates of polymorphonuclear leukocytes and macrophages that might obstruct capillaries, release free radicals, and damage endothelium in all organs.[246-248] Their role after CA has not been clarified. The roles of inflammatory mediators and endothelium-derived nitrogenous vasodilators, which play roles in septic shock, need to be conclusively studied as to their roles in brain damage after CA.[198,199]

IV. THERAPEUTIC CONSIDERATIONS

A. Guidelines for CPR and Cardiopulmonary-Cerebral Resuscitation (Figure 6)

Since the 1970s, one of us (PS) has written CPCR guidelines for the World Federation of Societies of Anaesthesiologists (WFSA).[1] The American Heart Association (AHA) guidelines committees have continued focusing on CA-ROSC by CPR, and on hospital discharge rates. Little attention has been paid to therapeutic potentials for saving cerebral neurons, although cerebral resuscitation starts with steps A-B-C of BLS, and continues through ALS and PLS[1] (Figure 6).

The AHA Guidelines 2000 Conference on CPR and Emergency Cardiovascular Care (ECC) updated previous recommendations[7] by emphasizing evidence from randomized clinical outcome studies as the basis for all new clinical recommendations. Such trials are considered "gold standard" for evaluating novel treatment potentials for subacute or chronic diseases (e.g., oncology, heart failure). We agree. We do *not,* however, consider them to be gold standard for evaluating physiologically effective cerebral resuscitation potentials.[2,15-21] Much more controllable are the results from clinically realistic outcome studies in animals high on the phylogenetic scale, such as dogs. The discrepancies between negative results in clinical trials and positive animal outcome data in acute medicine (e.g., for shock, brain trauma, sepsis, or stroke) can be explained by the limitations of randomized clinical outcome trials, which include:[2] Cases within the therapeutic window cannot be selected in the seconds available to initiate (novel) resuscitation; numerous variables that can influence outcome, particularly the initial insult, cannot be accounted for in the randomization nor can they be controlled during the trial; despite standardized protocols, there is inevitably a great variability in the timing and quality of life support between cases and centers; subgroup analyses may be revealing, but creation of

subgroups by post-randomization characteristics is considered by statisticians to be unreliable and may be misleading. It may be impossible to statistically document anything less than a consistent breakthrough effect. We know of no known method to overcome these problems, except to rely more on large-animal outcome data, and on "sequential trials" within regions with CPCR case registries. In those studies, one would compare standard therapy results of the recent past with those of a novel therapy for cases considered "unresuscitable" in the past. Clinical feasibility and side effects of novel treatments in sick people can be determined without randomization. Therefore, based on personal experience and the literature, one of us (PS) feels that novel cerebral resuscitation strategies that are simple and inexpensive, and that have shown clinically significant cerebral outcome benefit in several clinically realistic outcome models in animals high on the phylogenetic scale, should be taken, via clinical feasibility and side-effect studies (which are inexpensive), to general clinical use.

AHA committees base their recommendations of 2000[7] on the following categorization: Class I means evidence of benefit is excellent and the measure is acceptable, safe, and definitely useful. Class IIa means that the evidence is good to very good, and considered a choice by the majority of experts. Class IIb means that evidence is fair to good, useful as an alternative. Class "indeterminate" means that evidence is insufficient. Class III means that the treatment is unacceptable because of either documented harm or no documented benefit.

The newly designed AHA algorithm (Figure 2) seems to represent the consensus of panel leaders, but is not necessarily the opinion of the authors of this chapter. Novel brain-oriented therapies during and after CA, not included in the AHA recommendations of the 2000 conference, are included in the authors' recommendations for a CPCR system of 2000 (Figure 6).

Adult Basic Life Support

The AHA recommends that: rescuers confronted with a suddenly unresponsive adult should "phone first" (except in cases of submersion, trauma, and drug intoxication); volumes for bag-mask ventilation be smaller to prevent gastric inflation; the laryngeal mask and esophageal tracheal tube be acceptable; the pulse-check requirement for lay rescuers be eliminated; and that the chest compression rate be 100/min, with a compression:ventilation ratio of 15:2 for either one or two rescuers. For foreign body obstruction, abdominal thrusts (Heimlich maneuver) is retained for the still conscious victim, although efficacy is based on anecdotes. For unresponsive adults, lay rescuers do not have to treat foreign-body obstruction with an abdominal thrust. Sternal compressions may act as thrusts. Finger sweep of mouth and pharynx is retained. Use of an automatic external defibrillator (AED) by BLS responders (police, firefighter, security personnel, etc.) is a class IIa recommendation.

We agree with all of the above, except that we recommend to initiate steps A-B-C first, and call EMS as soon as possible, preferably by a second person. In out-of-hospital cases of sudden coma, fewer than 20% of resuscitation attempts so far have been started by a lay bystander.[22] This is now the weakest link of the life support chain.[249] Safar, Laerdal, and Eikeland have, since the 1960s, developed and documented the superiority of simplified self-acquisition of life supporting first aid (LSFA) skills by lay persons;[250] LSFA now should include CPR-BLS (steps A-B-C), clearing of foreign matter, AED, external hemorrhage control, positioning for shock or coma, "rescue pull," and calling the EMS system for help. We recommend as part of LSFA more emphasis on backward tilt of the head, the use of an AED (when available) immediately following initiation of steps A-B-C, and starting external cooling as feasible (Figure 6). We have recommended ongoing community programs, including media,

internet, and self-training systems in schools and driver's licensure stations. Self-training systems with individualized videotape-coached manikin practice were found to be more effective than standard courses by instructors.[250]

The AHA guidelines committee suggested that if the rescuer is unwilling or unable to perform direct *mouth-to-mouth ventilation*, he/she should at least perform chest compressions only, which may be more effective than doing nothing.[251] They did not mean to teach only chest compressions without ventilation. The AHA committee ignored the only *human data* on ventilation by sternal compressions alone.[36] That study showed no reliable ventilation, with or without tracheal tube. Also ignored were *dog data* showing that during CA, in the presence of airway obstruction, the still-oxygenated aortic blood will become rapidly deoxygenated under sternal compressions alone.[252] Animals have straight upper airways, but comatose humans' upper airways are kinked and obstruct without backward tilt of the head.[27,28] The AHA should not give up teaching *one* BLS approach, steps A-B-C, for simplicity (lay persons cannot diagnose the cause of sudden coma); and stress step A, airway control by backward tilt of the head.[253,254] This should be provided throughout sternal compressions by keeping the victim's chest and shoulders elevated to sustain spontaneous head-tilt (Figure 6).

Adult Advanced Life Support

Pharmacology. The AHA recommendations state (and we agree) that the supporting evidence in general for all drugs in use for ALS is only fair. All *anti-arrhythmics* are determined as class IIb.[7] References to bretylium have been dropped. Amiodarone is regarded as having better evidence-based support than any other anti-arrhythmic. Lidocaine remains acceptable as an anti-arrhythmic for countershock-refractory VF and pulseless VT, but is considered

"indeterminate." Magnesuim is recommended only for hypomagnesemia and "torsades de pointes" as class IIb.

Vasopressors remain important to cause peripheral vasoconstriction, which enhances ROSC by increasing coronary perfusion pressure.[48,49,255-257] Other vasopressors are also effective.[48-51,258,259] Cardiac stimulants without vasopressor effect do not enhance ROSC.[48,257] Escalating doses or high doses of epinephrine[21,50,186,260,261] are not recommended by the AHA for routine use during CA (indeterminate), although they enhance ROSC.[21] There is some evidence that cardiac arrest survivors who received high-dose epinephrine have more cardiac complications after ROSC.[21,260-262] Vasopressin (40 U i.v. as a single dose) may be substituted for epinephrine (1 mg i.v.) as an alternative vasopressor in VF or VT with CA (class IIb).[51,263] Interestingly, the AHA considered epinephrine as "indeterminate" due to the lack of placebo-controlled clinical trials. In patients, randomized withholding of epinephrine or an alternative vasopressor would be unethical, considering the powerful animal data in support of increasing coronary and cerebral perfusion pressures during steps A-B-C.

Buffer therapy to correct metabolic acidemia during CPR-ABC, although found beneficial in enhancing ROSC,[48,53-58,264-267] has remained controversial because of some suspicions of its being unnecessary or harmful.[7,56,57,268,269] Other recent data support the use of $NaHCO_3$, because tissue lactic acidosis depresses the myocardium and is associated with enhanced ischemic brain damage.[156] Tissue hypercarbic acidosis without hypoxia (correctable by perfusion and ventilation) may temporarily hamper function, but does not cause permanent damage to heart or brain.[164,165] One must differentiate between $NaHCO_3$ (or trif-buffer) administration before vs after ROSC. We recommend that before ROSC, during CPR steps A-B-C, $NaHCO_3$ 1-2 mmol/kg be given i.v. only when estimated arrest time or CPR time is long. In

these cases, buffer therapy can enhance ROSC. After ROSC, during acid washout, blood base deficit worse than about 10 mEq/L may depress the myocardium and worsen the cascades of encephalopathy, and therefore should be normalized with titrated $NaHCO_3$ i.v. CO_2 washout should be controlled by ventilation.

Airway. The AHA recommends[7] that tracheal intubation in unconscious patients be attempted only by experienced health care providers. In patients not in CA, emergency responders should confirm tracheal tube position with a nonphysical examination technique (e.g., esophageal detector device, end-tidal CO_2 indicator) (class IIa). In CA patients with low pulmonary blood flow, these devices are considered class IIb. ALS providers without regular field experience should use noninvasive techniques for airway management, such as pharyngeal tube and exhaled air ventilation via valved mask with O_2 enrichment[270] or laryngeal mask. Mouth-to-mask ventilation frees both hands for mask fit, head-tilt, and jaw thrust, which are more difficult to provide with bag-valve-mask ventilation.[29]

Defibrillation. Health care providers who perform CPR should be trained, equipped, and authorized to use an AED (class IIa). Hospitals should establish programs to achieve early defibrillation throughout their facilities (class I).[7]

Step C modifications.[7] To date, no pneumatic modification or adjunct has been shown to be universally superior to standard sternal compressions for prehospital closed-chest artificial circulation, in terms of blood flows produced and ROSC achieved. Weisfeldt et al[271] documented the chest-pump mechanism of blood flow produced by sternal compressions, i.e., overall intrathoracic pressure fluctuations with functional venous valving.[272] We now know that the chest pump mechanism prevails in keel-chested dogs, the heart compression mechanism in children and broad-chested dogs, and individual combinations exist in adult humans.[273]

Simultaneous ventilation/compression (SVC) CPR[37,271-275] requires tracheal intubation, which makes synchronizing not necessary. Intermittent or sustained abdominal compression (IAC) CPR[276-279] for in-hospital resuscitation the AHA recommends as an alternative intervention to standard CPR (class IIb). It improves blood flow and does not require intubation, but requires two operators. High-frequency CPR (>100 compressions/min)[280] lacks clinical studies and is considered as "indeterminate." Active compression-decompression (ACD) CPR[281,282] is considered as an acceptable alternative (class IIb). Vest CPR[283,284] may be considered an alternative for in-hospital use or during ambulance transport (class IIb). Mechanical (piston) CPR[285,286] is considered as an acceptable alternative in circumstances that make manual chest compressions difficult (class IIb). Phased thoracic-abdominal compression-decompression CPR lacks[287] clinical outcome data ("indeterminate"). The impedance threshold valve[288] for use with standard CPR is not recommended, but is acceptable as an adjunct to ACD-CPR (class IIb). Open-chest direct cardiac massage (OC-CPR)[1] can be considered under special circumstances, but should not be done simply as a late last-ditch effort (class IIb). Emergency cardiopulmonary bypass (CPB)[151] lacks clinical outcome studies and is considered "indeterminate." We would like to see OCCPR and CPB tried for very early application in cases of CA where SECPR-ALS of a few minutes fails to achieve ROSC.

Ventilation. Inflation volumes ($PaCO_2$), inhaled O_2 (FiO_2), and inflation pressures are under consideration.[7,289] The fear of gastric distension due to high inflation pressures seems exaggerated.[27] The fear that high FiO_2 (PaO_2) during ROSC attempts may worsen cerebral outcome by increasing reactive oxygen species (ROS) may be exaggerated.[290-292] The re-examinations of exhaled air vs air vs O_2 for ventilation during BLS and ALS, by *Idris* et al,[289] are

laudable; they should be extended to evaluating these effects on the postischemic encephalopathy.

B. Standard Brain-oriented Life Support (Table 3)

Early after ROSC, while the patient is unresponsive, hypotension, hypoxemia, hypercarbia, hyperthermia (even mild), hypoglycemia, and fluid and acid-base derangements must be avoided as they can worsen cerebral outcome.[2] Accurate control of these variables can mitigate the postischemic encephalopathy (Figures 3 and 4). The effects of these general measures and of novel cerebral resuscitation strategies to be described subsequently, require more intensive patient monitoring than is usually practiced for PLS after CA.

Early after CA, the progression of recovery from coma should be followed (usually in the ICU) by monitoring the Glasgow Coma Score (GCS) (Table 1).[1,293] The Pittsburgh Brain Stem Scale (PBSS) may also be determined (Table 1),[1,15-21] as it includes specific cranial nerve reflexes that have value for outcome prediction. *Later*, the patient's progress should be monitored in terms of overall performance categories (OPC) 1-5,[294] and, separately, cerebral performance categories (CPC) 1-5[1,15-21] (Table 2). These measurements have become standard international recommendations.[1,23] We added CPC to OPC because a patient can be severely handicapped by extracerebral organ dysfunction (OPC 3) while rational (CPC 1).

Standard brain-oriented life support by control of extracerebral organ function is covered only partially in the AHA guidelines,[7] and more detailed in the recommendations by the authors of this chapter[1,2] (Table 3). Normotension, normoxia, and normocarbia are self-understood. For control of MAP early after ROSC, a titrated i.v. infusion of epinephrine or norepinephrine may be more effective than infusion of phenylephrine, dopamine, or dobutamine. The latter may

have advantages later after CA. In cardiac failure later after CA, a spectrum of possibilities, from titrated dobutamine or norepinephrine to assisted circulation with CPB or aortic balloon pumping, is available. Throughout coma, controlled ventilation to at least 12 hours postarrest seems desirable to combat cardiovascular-pulmonary failure. To control "fighting" tracheal tube and ventilator, we favor use of low (softening, not apneic) doses of a relaxant, titrated i.v., to permit monitoring of neurologic recovery, to avoid over-curarization, and to allow titration of sedatives and narcotics. A hypnotic or narcotic agent should be titrated i.v. to control hypertension and mydriasis (sympathetic discharge). Corticosteroid therapy is controversial.[295] Optimal post-arrest levels for PaO_2, $PaCO_2$, base deficit, serum osmolality, and blood glucose are not yet clarified.

Prolonged hypoglycemia (blood glucose below 50 mg/dL) is deleterious to the brain.[143,296] Severe hyperglycemia present before and during GBI, in animal models, seems to worsen neurologic damage,[143,163,296] most likely because it increases brain lactic acidosis, which decreases brain pH.[156] During and after CPR, there is usually a spontaneous moderate hyperglycemic response which may be desirable. Three animal studies showed worsened outcome with glucose i.v. during or after reperfusion;[297-299] and other studies suggest improved neuronal recovery with hyperglycemia in focal ischemia,[300] in vitro hypoxia,[301] or after asphyxial CA,[111] using our group's asphyxial CA rat outcome model.[107-109] The last study by our group showed that postarrest *moderate hyperglycemia,* by glucose administration *plus insulin* (blood glucose of about 150 mg/dL), can improve functional and histologic cerebral outcome over glucose alone, insulin alone, or no treatment with moderate hyperglycemia without insulin.[111] In cases of prehospital CPR, high blood glucose levels at the time of arrival at the hospital correlated with poor neurologic recovery;[302-304] in one study, however, these hyperglycemic

patients were diabetic.[303] High glucose levels correlated with the duration of CPR attempts.[303] Immediately after acute stroke, hyperglycemia might be helpful[300] or harmful.[305] Because sudden coma can be caused by hypoglycemia, routine withholding of i.v. glucose after CA is debatable. We currently recommend reperfusion without added glucose and postarrest monitoring and titration of blood glucose levels to 100-200 mg/dL. Suspected hypoglycemia should be treated immediately with glucose infusion.

C. Cerebral Blood-Flow Promotion (Figure 3)

Support of hypertensive reperfusion can be found in papers documenting this treatment's ability to overcome the immediate no-reflow phenomenon,[167-179] to open highly resistant areas of the cerebral microcirculation,[171] to improve EEG recovery after prolonged GBI in cats,[81-84] and to have a variety of other positive physiologic effects in acute animal models.[81-84,130,132,168-175,183-186] In 1974, using a dog model of VF 12 min no-flow and external CPR, an immediate post-ROSC combination of norepinephrine-induced hypertension, intracarotid hemodilution with dextran 40, and heparinization improved outcome.[130] That was the first positive outcome study of cerebral resuscitation after CA. Heparinization plus thrombolytic therapy immediately after CA looks promising.[172,192-197,306] Thrombolysis would make sense to accompany the hypertensive bout because there is hypercoagulability[195] and suggestion of benefit in patients,[197] possibly for both heart and brain. In a recent dog outcome study, a brief hypertensive bout (MAP of 150 to 200 mm Hg for 1 to 5 min), followed by controlled mild hypertension, abolished evidence of immediate no-reflow[175] and correlated with improved neurologic and brain histologic outcome. A vasodilating endothelin antagonist[189-191] is only one possibility among many to provide CBF promotion after CA.

Induced hypertension has not undergone a prospective clinical trial, but after ROSC in patients, four retrospective patient studies have shown that brief (spontaneous or induced) arterial hypertension early after CA, was associated with good cerebral outcome, and/or hypotension with poor cerebral outcome.[307-310] One study in human CA survivors showed that good functional neurological recovery was independently and positively associated with arterial blood pressure after initial reperfusion, during the first 2 hours after CA.[309] In a recent retrospective study of over 1,000 CA patients,[310] good cerebral outcome was associated with higher SAP early after ROSC, also in multivariate analysis. Prolonged severe hypertension late post-arrest, however, might not be tolerated by the ischemic heart and might worsen vasogenic cerebral edema.[311] The effect of normovolemic or hypervolemic hemodilution alone[312] on cerebral outcome after CA is uncertain,[130-132,313-315] possibly because hematocrit values less than 25% can reduce arterial O_2 content below that compensated for by increased flow, and thus decrease O_2 delivery.[312] Post-CA cerebral hypoperfusion in dogs, however, could be prevented by hypertensive reperfusion plus normovolemic hemodilution with plasma substitute to a Hct of 20%.[175]

Our current recommendation for clinical use is in favor of a hypertensive bout as early as possible following ROSC. Indeed, this often occurs spontaneously, as a result of prior epinephrine administration. If not, it should be induced as early as possible after ROSC, by using a titrated i.v. infusion of a vasopressor. In dogs, norepinephrine by careful titration proved more effective than use of other vasopressors. Aiming for a systolic arterial pressure of 150-200 mm Hg for 1 to 5 minutes seems reasonable. After reperfusion for 15-30 min, a combination of Hct 30%, $PaCO_2$ 40 mm Hg, plus titrated moderate hypertension seems more beneficial for the brain than Hct 40% and $PaCO_2$ 30 mm Hg. After ROSC, during coma, monitoring mixed

cerebral venous PO_2 or SO_2 (in patients in the superior jugular bulb)[140,316,317] could guide titration of MAP, hematocrit, and $PaCO_2$[175,318] to keep cerebral venous PO_2 at > 25 mm Hg (SO_2 > 50%),[137-139,175-179] to avoid major global under-perfusion in relation to O_2 uptake. This will not detect multifocal hypoperfusion.

D. Open-chest CPR

In CA cases in which external CPR-ALS attempts of longer than 5 min fail to restore stable spontaneous normotension (usually patients with acute myocardial infarction), artificial circulation methods that are physiologically more powerful should be tried. Chest compressions raise venous (right atrial) pressure peaks almost as high as arterial pressure peaks[319] and increase ICP,[320,321] thus usually causing very low cerebral and myocardial perfusion pressures during "thoracic diastole."[322-329] Open-chest CPR (direct manual compressions of the ventricles) does not raise right atrial pressure, provides better cerebral and coronary perfusion pressures and flows than does external CPR in animals[320-332] and patients,[323] and achieves better ROSC and outcome in dogs[326] and patients.[333] Open-chest CPR, which was introduced clinically around 1900,[92,333] and used until 1960, inside hospitals, mostly for CA in operating rooms, yielded good clinical outcome results when applied promptly.[333] In four studies, the switch from external to open-chest CPR in patients, even out-of-hospital, has been shown to enhance the chance of ROSC, but has failed so far to increase the proportion of patients with good cerebral outcome.[334-338] This was to be expected because open-chest CPR has been initiated too late. The importance of early initiation of open-chest CPR was documented in an uncontrolled clinical study from Japan, where the rate of ROSC was highest in patients with early thoracotomy;[338] survival rate was 12% with open-chest CPR vs. 1% with conventional CPR. In a European study of mostly

asystolic patients,[336] out-of-hospital open-chest CPR, after very long futile external CPR, increased the rate of ROSC, but survival rate was only 6%.

In my opinion, medical students should learn open-chest CPR on dogs or pigs, as we have done in the 1950s, how to get your hand on the heart within 60 seconds, how to ventilate with IPPV plus PEEP, and how to perform open-chest defibrillation.[1,339] Trained physicians should consider switching to open-chest CPR much earlier than in the above-mentioned studies, perhaps even outside the hospital, and not only in victims of trauma.[1,2] Bystanders have not objected to open-chest CPR when it was tried in the field;[335,336] open-chest CPR attempts seem to be generally accepted (and admired) by the patients' families. In the hospital, if the stunned heart cannot be started up even with open-chest CPR, prolonged direct heart compressions can be performed with a mechanical device.[340] A method of minimally invasive direct heart compression, not requiring thoracotomy, is being evaluated.[341] In a recent pig study, this device was shown to be superior to standard external CPR in terms of higher coronary perfusion pressure and rate of ROSC (7/10 vs 2/10).[342] Open-chest CPR can be initiated rapidly and serve as a bridge to long-term CPB and definitive cardiac repair.

E. Emergency Cardiopulmonary Bypass

For cardiac arrest, emergency cardiopulmonary bypass (CPB), i.e., veno-arterial pumping via oxygenator, without the need for thoracotomy, was tried in dogs[343] and in patients[344] in the 1970s, but not pursued further.

Because CPB provides full control over blood pressure, flow, composition, and temperature, in the 1980s Safar initiated a systematic comparison in eight CA outcome studies in dogs,[151] comparing emergency CPB (by closed-chest veno-arterial pumping via oxygenator) with

SECPR-ALS. CPB provided greater cardiovascular resuscitability than CPR-ALS and thereby improved cerebral recovery.[90,151,345-350] In CA dog models, brief CPB provides controlled reperfusion that results in more reproducible outcomes.[345-351] Emergency CPB has been tried in hospital emergency departments for CPR-resistant CA cases.[352-354] Late initiation of CPB, and the excessive time (longer than 10 min) taken for cannulation of femoral vessels,[352] have so far led understandably to disappointing results. A more rapid method of vessel cannulation is needed for use by ambulance physicians, to use a portable CPB device for ultra-ALS to be initiated in the prehospital setting.[355-357] CPB might be initiated more rapidly via emergency thoracotomy.

When, during prolonged CPB, the heart is in intractable asystole or VF, the need for decompressing the left ventricle or the pulmonary circulation is debatable. Closed-chest CPB during VF of up to 8 h proved possible in dogs.[358] Experimental VF and closed-chest CPB in sheep with inadequate decompression of the left heart caused an increase in left heart filling pressure, left ventricular pressure, and pulmonary artery pressure; and lung failure.[356-358] Decompression of the left ventricle in sheep was achieved by keeping the pulmonary valve open with a spreading catheter (a helical spring).[359-362] In humans, after normothermic CA, in some cases, closed-chest CPB without the need for venting the left ventricle was sometimes applied for up to 5 h, and even for a day, without causing pulmonary edema.[358,363,364]

Prolonged emergency CPB[151,358] or open-chest mechanical cardiac massage,[340] for many hours, could give the stunned heart a chance to recover from reversible cardiac failure or could be a bridge to coronary angioplasty, bypass procedure, insertion of a left ventricular assist device, or emergency heart replacement. When brain death is determined, prolonged CPB could become a bridge to organ donation. These possibilities make present guidelines for

discontinuing CPR in the field after a failed ROSC attempt of 30-60 min as being too pessimistic.[3,365-367] Emergency (portable) CPB would give presently "unresuscitable" sudden cardiac deaths with "hearts and brains too good to die" (Beck-Safar) a new chance. These cases amount to up to 50% of out-of-hospital CPR attempts.[1,7,8]

Hyperbaric oxygenation (OHP, HBO) as a possibility for enhancing the recovery of the brain after CA and ROSC is a separate topic, beyond the scope of this chapter. OHP makes physiologic sense, lacks convincing outcome data so far for use after CA, and is logistically too problematic for use in emergency resuscitation.

F. Pharmacologic Strategies (Figure 4)

Results with the pharmacologic cerebral resuscitation potentials for CA, explored so far[2,368,369] have paled in comparison with hypothermia (see subsequently). Historically, in 1976, the Pittsburgh group began to devote 10 years to evaluating the efficacy of *thiopental loading*, starting with GBI in monkeys,[370] using for the first time a reproducible long-term intensive care outcome model[103] to determine the neuron-saving potential of a drug administered *after* reperfusion. Thiopental caused a significant reduction in post-arrest neurologic deficit and morphologic brain damage.[370]

The choice of *barbiturate* as the first drug tested (in a plan to explore many drugs) was based on positive results with use of barbiturates for experimental ischemic stroke,[371-373] for incomplete ischemia,[374] and for protection before global ischemia.[375] Because no other reliable animal outcome model was available at the time, a second study with more accurate blood pressure control was conducted in monkeys with GBI, by the same group.[376] This failed to duplicate the outcome benefits of the first study.[370] In a cat VF model,[377] barbiturate suppressed seizures.[378] Several beneficial mechanisms justified barbiturate trials.[2,379-382] Subsequent studies by others also gave mixed results.[378,383,384] Because of the uncontrolled clinical use of barbiturate loading at that time, and promising clinical feasibility trials,[153] a multicenter clinical trial was conducted.[15] In this Brain Resuscitation Clinical Trial (BRCT I, 1979-84) the proportion of patients with good cerebral outcome was statistically the same in thiopental and control groups, but a subgroup with long arrest or CPR times showed a trend toward better cerebral outcome after thiopental treatment. Since barbiturate loading proved hazardous for the cardiovascular system,[15] we have recommended that anesthetic (not loading) doses should be titrated after CA to control seizures or intracranial hypertension. However, even smaller doses

than those that silence the EEG can be expected to be somewhat beneficial.[385] Barbiturates suppress active cerebral metabolism to maximally 50% normal, but not basal metabolism which is also suppressed by hypothermia. Since we saw microinfarcts after GBI, we hypothesized that a beneficial effect can be expected. The cognitive dysfunction in some patients after cardiac surgery with CPB, probably mainly the result of microinfarcts, was mitigated with barbiturate.[386] A barbiturate combination treatment in dogs after CA seems to give some cerebral benefit.[387] Present conclusion is that some barbiturate treatment protocols can benefit the brain, but only if cardiovascular-pulmonary complications are avoided.

Calcium entry blockers seemed promising also because of multiple beneficial mechanisms, including mitigation of cerebral hypoperfusion after CA.[388] We found an i.v. infusion of lidoflazine after CA to significantly reduce neurologic damage in dogs.[389] The same was found with calcium entry blocker nimodipine in our GBI monkey model.[390] In the second major clinical trial (the BRCT II, 1984-89)[17-20] post-CA lidoflazine also failed to achieve a significantly higher proportion of patients overall with good cerebral outcome compared to the placebo group, but a subgroup of patients without postarrest hypotension or re-arrest did achieve a significant benefit.[20] A similar clinical study of nimodipine, in Helsinki, also failed to show a statistically significant overall improvement, but in subgroups with prolonged CPR times, nimodipine had a significantly higher proportion with good cerebral outcome.[391]

We do not recommend the routine use of barbiturate loading or calcium entry blocker therapy after CA, because in both major clinical trials these drugs were difficult to manage. Their use was associated with a higher incidence of hypotension and re-arrest. For managing surgical anesthesia or sedation in the ICU after CA or TBI, the optimal agents remain to be determined. Barbiturates and halogenated inhalation anesthetics[392-397] look better than opiates,[398]

which in large doses are excitotoxic.[398,399] One cannot, however, assume that a slight benefit in a rodent focal brain ischemia or TBI model translates into benefit after CA in dogs or patients.

Many other drugs with suspected cerebral resuscitation potentials have been studied in neuron cultures, rat hippocampus slice models, and (incomplete) forebrain ischemia rodent models. In some there was a suspicion of benefit. When studied in larger animals *after* CA or GBI, with appropriate temperature controls, however, outcome data were unconvincing or only suggestive in terms of cerebral benefit. There have been no more randomized clinical outcome studies with drugs after CA. Drugs explored for administration after ROSC, in animals, and found to possibly do some good, but without a breakthrough effect in clinically relevant large animal outcome models, include: phenytoin,[400,401] the anti-oxidant tocopherol (Safar et al, unpublished), the excitatory neurotransmitter NMDA receptor blocker MK-801,[402-404] the AMPA receptor blocker NBQX,[405,406] the aminosteroid trilizad,[407,408] the neuron-specific calcium entry blocker SNX-111,[409-413] lidocaine[414,415] (Safar et al, unpublished), various other calcium entry blockers (some of which are not available for i.v. use in the US);[416-429] fructose biphosphate;[430-432] the good gene bcl-2;[433] insulin with glucose and moderate hyperglycemia;[111,434] estrogen;[435] a PARP inhibitor[436] (see Figure 4); and, of course, lessons to be learned about possible intravascular clotting after CA as established for stroke.[437,438] Unconvincing results in large-animal outcome models[2] were often preceded by beneficial effects on mechanisms and even on histologic damage of the hippocampus in rodents. Some of these drugs were expected to fail, either because uni-mechanistic effects cannot mitigate the multifactorial pathology (Figure 4), or because they fail to penetrate the blood-brain-barrier, which is not grossly damaged after CA of up to 30 min no-flow.[369] Drugs given at the start of ischemia might be more effective. Even there, however, of 14 drugs we explored for preservation by aortic flush at the start of CA 20 min

in dogs,[369] 13 drugs failed to improve cerebral outcome, while mild hypothermia normalized outcome. Only the anti-oxidant tempol, which is water soluble and penetrates the blood-brain-barrier, was found to give benefit, and that only when present during ischemia (preservation), but not when given for resuscitation after CA (see below, "Suspended Animation").[439] Other anti-oxidants which do not penetrate into the brain may have beneficial effects on the microcirculation,[440-442] but not on neurons.[443-445] Mechanistically intriguing is the discovery of mitochondrial transition pores (MTP) late after GBI, as a culprit in causing DNA damage through calcium flux.[446] This mechanism could be mitigated with cyclosporin-A, but only if the blood-brain-barrier is broken in GBI with a needle puncture[447] or in focal ischemia;[448] but not after temporary GBI with the blood-brain-barrier intact.[369]

Combination treatments would make more sense than drug effects with single mechanisms (Figure 4). Many combination treatments remain to be evaluated, but a practical method to accomplish this is still elusive. Drugs that seem to have a slightly beneficial effect after an insult other than CA, such as MCA occlusion, should not be expected to benefit the brain after GBI. Other limiting factors are solubility, toxicity of vehicle (such as DMSO), methemoglobinemia (as with tempol), and exorbitant costs of some experimental drugs. For more references on the exploration of pharmacologic strategies in various brain insults, see reviews.[2,368,369]

G. Hypothermic Strategies (Figure 5, Table 4)

Accidental exposure hypothermia causing loss of limb or life has been described throughout human history. So has the use of therapeutic local application of cold to reduce inflammation and pain. Therapeutic whole body cooling was first used empirically in the 1940s

for cancer pain and brain trauma in patients.[449] The history of *therapeutic* hypothermia, began in the 1950s with elective moderate hypothermia of the brain, introduced under anesthesia, for the protection-preservation during brain ischemia needed for surgery on heart or brain. All began with the pioneering work of Bigelow,[450,451] Dripps,[452] Rosomoff,[453-458] and Negovsky.[95,459] In the 1960s, R. White and Albin[460-470] pioneered the experimental documentation of local hypothermia after spinal cord injury. When induced *before* CA (protection), moderate hypothermia (28-32°C) can preserve the brain *during* no-flow of up to 20 min.[450-452] *Deep* hypothermia (16-27°C) causes VF or asystole but, when induced and reversed by CPB, preserves brain viability during 30-45 min no-flow, whereas *profound* hypothermia (5-15°C) could preserve the viability of brain and organism for at least one hour of total circulatory arrest in dogs, and for 45 min arrest in patients. These trials in large animals[471-483] and patients[484-488] were under anesthesia, with slow, elective induction of hypothermia by surface cooling or rapid cooling with CPB.

Lessons were learned from experiments and clinical data on *drowning*.[489-494] One must differentiate between near-drowning with circulation (pulse) continuing, when the main injury is that of the lungs; and full drowning, i.e., to clinical death (no pulse). The "miraculous" recoveries of humans after ice-water drowning (with asphyxia) or exposure hypothermia (without asphyxia),[495-498] from estimated CA times of up to 1 hour, can be explained by the fact that the brain reaches protective hypothermic levels before the heart stops.[345,499] This is in contrast to severe brain damage after even brief CA as the result of normothermic asphyxiation or normothermic drowning.[490-494]

Moderate resuscitative (post-insult) hypothermia (30°C) after CA was explored already in the 1950s in animals[500,501] and patients.[458,502-504] The summary of the physiology of

hypothermia by Dripps and Severinghaus of 1956[452] is a classic. Safar included resuscitative hypothermia in 1961 as one step in his recommended CPCR system.[25,26] Resuscitative hypothermia research was then given up for 25 years, probably because of uncertain benefit, management difficulties, and fear of side effects (VF, coagulopathy, infection). In the early 1980s, disappointed with drug treatments,[2,15-21] Safar's group revived research into resuscitative moderate hypothermia (30°C) after CA.[505-507] Outcome benefit was modest. The subsequent discovery by Safar's group in dogs in 1987 that *mild* hypothermia (33-36°C), which is simple and safe compared to moderate hypothermia, is protective-preservative,[74,151] was followed by documentation of resuscitative effects on the brain after prolonged CA;[508-515] and after asphyxial CA in rats.[516] Simultaneously, with these dog studies other investigators discovered mild protective, preservative, and resuscitative hypothermia in incomplete forebrain ischemia rat models.[517-524] All this rekindled widespread hypothermia research in the 1990s. The neuroscientists in Miami, Lund, and Detroit who used rats documented the ability of mild resuscitative hypothermia to reduce hippocampal histologic damage. They also examined many biochemical mechanisms. Now, mild cerebral resuscitative hypothermia after prolonged normothermic CA and ROSC, remains at the cutting edge of reanimatology.

External cooling of the conscious organism causes potentially hazardous shivering and a sympathetic discharge with vasoconstriction, arrhythmias, and thermogenesis, through the *homoiothermic* defenses dictated from the hypothalamus.[525] *Internal* cooling of blood is less likely to cause shivering, even in the conscious organism. In order to make hypothermia therapeutic and safe, these defenses must be blocked and *poikilothermia* achieved, either by the insult (ischemia or trauma) suppressing the temperature regulating hypothalamus-pituitary system, or by drugs (sedatives, anesthetics, relaxants).[526] When some normothermic mammals

hibernate they release a still putative hibernation induction trigger[527] or a "hibernation specific protein" (HSP)[528] from the liver to induce poikilothermia. That lowers body functions parallel with body temperature dropping to 20-30°C in cool environments, without tissue hypoxia. Resuscitation researchers can probably learn more from the physiology of profoundly hypothermic hibernating turtles with tissue hypoxia.[529]

Risks of moderate hypothermia, even with poikilothermia, include arrhythmias;[452,530] coagulopathy as a result of reversible platelet sequestration and depression of coagulation enzymes;[452,531-534] and – if prolonged – pulmonary infection,[452,535,536] at least when life support is not ideal. Deep-to-profound hypothermia slows the microcirculation and causes myocardial depression, hypotension, arrhythmias, and CA at 22-27°C.

Moderate resuscitative hypothermia, induced immediately *after* an insult, when first studied around 1960, suggested benefit in dogs with focal brain ischemia[455] or brain contusion,[457] and yielded unconvincing results after CA in dogs and in patients.[501-504] It was discontinued, probably because of clinical management problems and perceived risks. In the early 1980s, resuscitative hypothermia research for CA was revived, using reliable, clinically relevant large-animal outcome models.[505-515] *Moderate* hypothermia (30°C) gave borderline benefit for the brain, but had side effects for the heart.[507]

In 1987, at a conference in Pittsburgh, Hossmann[84] reported that in cats with GBI there was a correlation between *mild* (unintentional) precooling and enhanced EEG recovery. At the same meeting, Safar[74,151] discovered a correlation between good cerebral outcome and *mild* (unintentional) hypothermia (34-36°C) present at the onset of VF in dog experiments. This led to a systematic series of five major outcome studies in dogs of prolonged normothermic CA

followed by *mild resuscitative* cerebral hypothermia (34°C), induced *immediately after* reperfusion and maintained for 2-3 h[508-511] or 12 h[512] (Figure 5).

In the *first* study,[508] VF 12.5-minute no-flow was accompanied by head immersion in iced water (which reduced brain temperature by only 1°C) and followed by reperfusion cooling with brief CPB to 34°C. Functional and morphologic brain outcome variables were significantly improved in the hypothermic groups.

In the *second* study,[509] VF 10-minute no-flow was reversed by standard external CPR; mild hypothermia induced within 5 to 10 min after reperfusion achieved the same significant improvement as in the first study. Cooling was performed with a clinically feasible but complex combination of head-neck-trunk surface cooling, plus cold fluid loads administered intravenously, intragastrically, and nasopharyngeally.

In the *third* study,[510] VF 12.5-min no-flow, brief CPB, and immediate mild (34°C) or moderate (30°C) hypothermia improved functional and morphologic brain outcome, but deep postarrest hypothermia (15°C with CPB) did not improve function and worsened brain histology. This contrasts with the greater brain protection (with prearrest induction) achieved with deep, as compared to moderate, hypothermia. Moderate or deep postarrest hypothermia also worsened necrotic foci in the myocardium. It could worsen reperfusion.

In the *fourth* study,[511] with the same model as in the first study, a 15-min delay in the initiation of mild cooling after normothermic reperfusion offset the mitigation of functional deficit and decreased the mitigation of histologic damage, compared to the more effective immediate post-ROSC cooling. In studies 1-4, even dogs with complete functional recovery had some histologic brain damage.

In the *fifth* study of normothermic VF-CA 11 min,[512] a combination treatment, of mild hypothermia for 12 h and CBF promotion, led to the best outcome yet encountered in dogs (Figure 5). We compared a control group 1 (normothermic standard therapy) with a combination treatment group 2, which received mild hypothermia by head-neck-surface cooling plus peritoneal instillation of cold Ringer's solution to keep brain temperature 34°C from reperfusion until 12 hours. In addition, group 2 received CBF promotion by induced moderate hypertension (MAP 140 mm Hg) to 4 hours, colloid (dextran 40)-induced reduction of Hct from 40% to 30% for 12 hours, and $PaCO_2$ of 40 mm Hg (instead of 30 mm Hg as in control group 1) from 3 hours to 20 hours. At 96 hours after resuscitation, all eight dogs in control group 1 remained severely damaged, while six of eight dogs in treatment group 2 had recovered to functional normality. The histopathologic damage scores in the treatment group were the lowest ever achieved. Final 96-hour overall performance category, neurologic deficit scores, and brain histopathologic damage scores showed highly significant group differences ($p < 0.001$). Also significant was the difference between the outcome in treatment group 2 of this study versus outcomes in previous studies using the same model and comparable insult, with CBF promotion alone[132] or mild hypothermia alone.[511] The control group had the same poor outcome results as in 50 control experiments with a comparable insult and the same model in the past. We recommend clinical trials of a combination treatment protocols based on experimental group 2 in this study.[512] Mild cooling in all five studies caused no cardiovascular or other side effects. We found in our asphyxial cardiac arrest rat model – as expected – that mild protective-preservative hypothermia reduces brain damage better than resuscitative hypothermia.[516]

The *mechanism* by which mild hypothermia protects and resuscitates is multifactorial.[2,508] The ability of hypothermia in normal brain to reduce $CMRO_2$ by about 7% per °C alone (in the

absence of shivering), in animals[453,537-540] or humans,[122] cannot explain the resuscitative effect of mild hypothermia. The temperature coefficient Q10 expresses $CMRO_2$ at one temperature divided by $CMRO_2$ at 10°C lower. A Q10 of 2 expresses a 50% reduction in $CMRO_2$, as it occurs in the normal brain between about 38°C and 28°C. Then, with artificial circulation, a Q10 of 5 expresses a reduction in $CMRO_2$ from 50% to 10% between 28°C and 18°C. The latter is caused by depression of basal metabolism.[541] *After* CA, however, *mild* hypothermia seems to have no significant effect on CBF and $CMRO_2$.[177,179]

The beneficial *mechanisms* include preservation of ATP,[145,146] mitigation of abnormal ion fluxes;[147] reduction of lactic acidosis,[542] free fatty acid production,[543] and excitatory neurotransmitter release;[543,544] slowing of destructive enzymatic reactions by 1.5% per °C (Arrhenius effect); protection of lipoprotein membrane integrity (assumed);[223] reduced edema and leukotrienes;[545] improved glucose utilization; slowing of free radical reactions;[546,547] reduced protease activity,[548] inflammation,[548,549] and stress protein formation;[550,551] and protection of the blood-brain-barrier.[552] The possibility of hypothermia mitigating "apoptosis" (acceleration of naturally programmed cell death), triggered by ischemia-induced DNA damage,[230-240] remains to be examined in clinically relevant CA studies in phylogenetically high species.

Is the benefit permanent? The benefit of intra-ischemic hypothermia on neuronal death is indeed permanent.[553] Brief (4 h) mild hypothermia after normothermic incomplete forebrain ischemia in rats postponed but did not permanently salvage hippocampal neurons at 2 months.[554] Delay and duration of hypothermia seem to be of critical importance. In gerbils, moderate hypothermia (32°C) initiated 1 h after the insult and sustained for 24 h was highly resuscitative in terms of behavior and histologic damage at 30 days;[555] while neuroresuscitation was less when hypothermia was initiated 4 h after the insult.[556] Increasing the duration of hypothermia to 48 h

resulted in long-lasting preservation of neurons at 1 month, even when initiation of hypothermia was delayed to 6 h.[557] The long-lasting effect of delayed (6 h), prolonged (48 h) hypothermia (32-34°C) on functional and histologic outcome at one month was confirmed in rats.[558] Our dog experiments with 1-2 h of profound hypothermic CA, followed by mild hypothermia for 12 h, led to OPC 1 and normal cognitive function many months later (see "suspended animation").

Cooling methods available at present are not ideal for clinical use (Table 4).[2,509,559,560] With normal circulation, brain and core temperature equilibrate rapidly. Cerebral hyperthermia adds injury to the ischemic or traumatized brain,[561-566] while normal brain can tolerate up to 42°C for up to 1 h.[567,568] Gradients between warm, focally injured brain and core body temperature can be 3°C. Thus, mild systemic hypothermia can prevent local cerebral hyperthermia.

Clinical methods for rapidly inducing mild cerebral or whole-body hypothermia in acutely comatose patients, outside and inside hospitals, are now under development (Table 4). They are plagued by secrecy due to commercial (patent) considerations. Whole-body immersion in ice water is rapid, but impractical.[452] Surface cooling by evaporation or ice bags is slow,[569] particularly under shock with vasoconstriction.[570] Peritoneal cooling is fairly rapid.[571] In dogs, nasal lavage with ice water achieved only slowly mild cerebral (and systemic) hypothermia.[509,572] Gastric-, rectal-, and i.v. cooling are also adjunctive.[509] Veno-venous or arterio-venous extracorporeal blood-shunt cooling is rapid[560] and may become the most practical for the induction of whole-body mild hypothermia, even out-of-hospital. Vena-cava cold rod intravascular cooling, as well as transpulmonary cooling with oxygenated very cold fluorocarbon solution, perhaps even with ice slush, are under evaluation by others. Isolated head-brain cooling would be ideal, thereby preserving the brain with moderate to deep hypothermia while protecting spontaneous circulation by keeping core temperature above 32°C.[463-470,559,573-584]

Several attempts at selective brain cooling by head-neck-surface cooling were successful only in small children and animals. Cooling the brain through cold infusion into a carotid artery would be the most rapid method. That – outside hospitals – would require common carotid cannulation, which is feared by some neurologists. Selective perfusion of the carotid artery with cold fluid or blood most rapidly induces deep to moderate cerebral hypothermia. In one study in baboons,[576,577] perfusion of one carotid artery from the femoral artery, with fluoroscopic guidance, with blood pumped via a cooling water bath, rapidly achieved moderate to deep bilateral cerebral hypothermia with minimal systemic cooling and without cardiovascular instability. Cold perfusion of the brain arteries via an aortic balloon catheter or "cobra-catheter" inserted via the femoral artery, is under evaluation (Cardeon Co.). Systemic blood shunt cooling via hemodialysis coil was slow.[585] During CA, most rapid is aortic cold flush (see later, Suspended Animation), or CPB with heat exchanger, once vessel cannulation is accomplished.[507-515]

Clinical implementation must consider that mild hypothermia (34°C) benefits the brain, moderate hypothermia (28-30°C) might induce VF, and mild cerebral *hyper*thermia (which can occur in brain-injured patients even with normal core temperature) is deleterious for the injured brain. Therefore, we recommend that all comatose patients have immediate monitoring and control of brain temperature (Tty or Tnp), as well as heart temperature (Tes or Tcv or Tpa or Tu). Shivering and vasospasm, if not absent because of post-anoxic coma or brain trauma, should be prevented with muscle relaxant and meperidine, diazepam, barbiturate, or other sedative. Although a 15-min delay in achieving mild hypothermia after reperfusion decreases the effectiveness,[511] even much later induction of mild cooling might have some beneficial effect on the brain which is permanent if prolonged.[555-558,586-595]

Clinical trials of mild hypothermia after prolonged CA followed the dog studies and are all positiive.[589-595] Exploratory studies in Japan[593-595] have been followed by randomized clinical outcome trials on 273 patients in Europe,[589,590] and 77 patients in Australia.[591,592] The data submitted are positive for mild hypothermia in all three clinical trials, although slow surface cooling was used, with the desired Tpa reached only after 1.5-6 h. In the Australian study,[592] 49% of hypothermic vs 26% of normothermic patients achieved good cerebral outcome (CPC 1 or 2); in the European study[590] the difference was 55 vs 39% ($p = 0.006$). There were no group differences in complications. The benefit of even more delayed and risks of longer lasting mild hypothermia after CA in dogs and patients need to be determined.

Rewarming[596,597] from mild to moderate hypothermia should be slow, perhaps once spontaneous circulation is restored, not faster than 1°C per hour. In CA, when the goal is ROSC, deep or profound hypothermia may need to be reversed to moderate hypothermia more rapidly, using CPB or CPCR.

For cerebral *resuscitation* from normothermic GBI in monkeys, a *combination* of moderate hypothermia and barbiturate gave a modest beneficial effect.[505,513,514] It was logical to explore drugs that proved ineffective at normothermia, to perhaps add benefit to mild hypothermia (Figure 5). We found suggestive evidence in dogs that post-CA neuron-saving is progressively enhanced by adding to mild hypothermia and hypertensive reperfusion,[512] thiopental and phenytoin,[513] and more so by including methylprednisolone and anti-oxidants.[514] The combination of thiopental loading and magnesium sulfate caused serious blood pressure problems. The combination of low-dose thiopental, phenytoin, magnesium sulfate, and mild hypothermia gave significant improvement over individual measures. Further improvement was seen by adding CBF promotion with moderate hypertensive hemodilution with sagittal sinus PO_2

normalization.[515] None of these treatments, however, gave a breakthrough effect comparable to that of mild hypothermia (Figure 5).

The discovery of mild hypothermia in dogs after CA has triggered increasing interest in its resuscitative application for *other insults*.

Acute stroke can also benefit from hypothermia. Post-insult *mild to moderate* hypothermia has been shown to be beneficial after experimental focal ischemia in dogs[454,455] and rats,[598-602] and is beginning to be investigated in patients.[603-606] In the future, earliest out-of-hospital initiation of selective brain cooling (e.g., arterial-carotid) by emergency physicians might preserve viability during transport, CT evaluation, and thrombolysis.

Traumatic brain injury benefits not only from normoxia[607] and normocapnia,[608] but also from moderate hypothermia after experimental brain contusion in dogs[456,457] and rats,[609-615] and after epidural brain compression (brain trauma) in dogs.[616,617] For prevention of posttraumatic intracranial hypertension in dogs, which can lead to herniation and brain death, moderate hypothermia (31°C) was more effective than mild hypothermia (35°C).[616] The first well-controlled randomized clinical trial of resuscitative mild to moderate hypothermia after TBI was conducted at the University of Pittsburgh. That study showed more patients with good outcome in the treatment group.[618] A subsequent multicenter randomized clinical trial in TBI patients, coordinated out of Houston, showed no overall benefit.[619] This can be explained by 8 hours delay to achieve 33°C, inadequate life support, and other flaws.[620] Recent guidelines for resuscitation from TBI[621] ignore hypothermia for ICP control. Other important considerations for the management of TBI cases are beyond the scope of this chapter.[622-629]

Spinal cord injury has been shown already in the 1960s, in animals, to benefit from local cord cooling; this could prevent or mitigate paralysis in a most dramatic way.[462,468] Because of colleagues' conservatism, this has not been taken to patients.[630]

Traumatic hemorrhagic shock, i.e., hypovolemic hypotension, requires preservation not of the brain, which protects itself with vasodilation,[134-137] but rather of the abdominal viscera, which vasoconstrict. Mild hypothermia during severe hemorrhagic shock in rats increases survival time and rate.[631-639] Mild titrated hypothermia for septic shock should be explored.[640,641] Deleterious cytokine reactions and ischemic tissues may benefit from titrated cooling, but inflammation to combat infection may be worsened.

Myocardial infarction (with spontaneous circulation) should be included among potential indications for mild hypothermia to be studied in animals and patients. It looks promising for reducing infarct size[642,643] which must be balanced against arrhythmogenicity of cooling to < 33°C.

A challenge lies in very rapid induction of cerebral hypothermia (already out-of-hospital) in conscious patients[560,606] with shock, stroke, or myocardial infarction -- without producing shivering. Shivering can be prevented or controlled with titrated i.v. sedation, as for example using meperidine (demerol, pethidine).

H. Suspended Animation

For (temporarily) unresuscitable CA, such as CA in combat casualties who exsanguinate internally to death over a few minutes, Safar and Bellamy in 1984 recommended research into "suspended animation" (SA), i.e., rapid preservation of the organism, to buy time for transport and repair under CA of 1-2 hours, to be followed by delayed resuscitation.[644] In such cases, CPR

plus infusions would be useless before surgical hemostasis. Civilian cases of traumatic exsanguination CA have had a near 100% mortality so far, in spite of emergency room thoracotomy.[645] A totally new approach is needed: suspended animation for delayed resuscitation. Preservation must be induced before loss of brain viability, which means before 5 min normothermic no-flow. Resuscitation will have to be with CPB.

In the late 1980s, Safar's group under Tisherman developed and used a new dog model of normothermic hemorrhagic shock followed by exsanguination to CA.[646-648] Cooling to profound hypothermia with CPB and heat exchanger, to Tty 10°C,[479] gave better outcome than cooling only to deep hypothermia (15°C).[478] Profound hypothermia during CA of 2 h resulted in survival with brain damage,[479] while profound hypothermia with CA of 1 h resulted in complete recovery of the brain in terms of function and histology.[483] Cerebral recovery was not influenced by lack of anticoagulant,[481] use of the University of Wisconsin organ preservation solution,[480] or moderate differences in diluted hematocrit.[482]

Profound hypothermia, induced electively with CPB, followed by circulatory arrest up to 1 h, has been survived to consciousness before in dogs[471-477] and in patients undergoing open-heart surgery.[484,485] Needed for SA are rapid emergency induction of hypothermic preservation, and a systematic search for the limits of no-flow time with reliable functional and histologic studies. Also, CPB by medics in the field is not feasible. Safar's group therefore explored the use of an aortic balloon catheter, which might be inserted rapidly into casualties at onset of coma, via thoracotomy, from the groin, or parasternally.[649] An aortic balloon catheter, inserted via the femoral artery, has been tried to increase coronary and cerebral perfusion pressures during sternal compressions, and to flush brain and heart with special solutions to enhance ROSC.[650-653] A rapid vessel-access method for use in the field must be developed. This would

enable flushing (upon onset of apnea, i.e., CA) an appropriate cold (perhaps medicated) solution, first into brain and heart, and then into the viscera.

Cold aortic flush for SA. The objective is to immediately induce preservation of brain and heart during the end-stage of bleed-out, within 5 min no-flow. The ultimate goal is to preserve the organism first for at least 30 min no-flow, and when more fluid or CPB becomes available, to lower Tty further to 5-10°C, to gain 1-2 hours of preservation. In exsanguination CA outcome experiments to 72 h in dogs, aortic arch flush at CA 2 min with normal saline solution (NSS) at ambient temperature (24°C) lowered Tty to 36°C and achieved cerebral recovery after CA 15 min.[654] A flush of NSS at 2°C, which lowered Tty to 34°C, achieved complete cerebral recovery after CA 20 min.[655] To achieve complete cerebral recovery with histologically normal brains after CA 30 min, Tty 25-30°C was needed, and the 2°C flush of NSS, in very large volumes, had to be introduced into the abdominal aorta to prevent ischemic damage to spinal cord and viscera as well.[656] Large aortic flush volumes require a second cannula at the venous side, as overflow must be drained or recirculated. This method could lower Tty by about 3°C per minute of flushing. Finally, Safar's group has recently achieved survival without neurologic deficit in dogs after exsanguination CA of 60, 90, and 120 min, using protracted aortic flush of NSS 2°C until Tty was 10°C.[657,658] That required, however, about 0.5 L/kg flush solution. That method would be feasible, in trained hands, where electric power and cold storage of very large volumes of fluid are available, as in major trauma hospitals' emergency departments. Clinical feasibility trials are being planned for SA via emergency thoracotomy, on exsanguinated trauma patients who are pulseless in the emergency department. Once CPB is available, preservation time could be extended further by asanguineous profound hypothermic low-flow.[659] Clinically, before more rapid vessel access is available with the chest

closed, emergency thoracotomy would give quick access to aorta and right atrium.[660] For profound hypothermic SA of 1-2 h in trauma cases, laboratory clarifications are needed on the effects of tissue trauma and coagulopathy (from ischemia, trauma, hemodilution, anticoagulants, and hypothermia).

Medicated aortic flush for SA. Combat medics would not have large volumes of cold fluid available. We therefore systematically explored aortic flush induction for preservation during CA 20 min no-flow,[661] using a small (portable) volume of NSS at ambient temperature (24°C), reinforced by pharmacologic preservation potentials. Without drugs, this led to survival with severe brain damage. In mini-series, 14 different drugs, one at a time, were tested as to outcome effect, following six pharmacologic strategies: delaying energy failure (adenosine, thiopental, fructose biphosphate), protecting ion exchange through depolarized membranes (phenytoin, MK-801, nimodipine), inhibiting proteases (no drug available), inhibiting apoptosis (cycloheximide, calmoduline antagonist W-7), protecting mitochondrial permeability pores (cyclosporin-A), and combating reoxygenation injury (tempol). None of the 14 drugs, in various doses, and with mild cerebral hypothermia, gave consistent OPC 1 (Table 2) after CA 20 min in dogs.[661-665] The anti-oxidant, tempol, which in aqueous solution permeates the blood-brain-barrier, looks promising when given in high doses added to the flush.[665]

SA strategies may not only be an answer to military and civilian trauma cases with presently unresuscitable CA. Some victims of normovolemic out-of-hospital sudden cardiac death may also benefit from SA, used to "buy time." At present, an estimated 50% of out-of-hospital CPR attempts in the U.S. (over 200,000 cases per year) are given up because ROSC is not achieved with standard external CPR-ALS. Aortic cold flush to profound hypothermic CA by emergency physicians in the field, or mild-to-moderate hypothermia with continued CPR-

BLS-ALS during transport, might bridge the patient over 30-60 min, with cerebral viability not lost, to initiate prolonged CPB in the hospital emergency department. CPB could then be continued for hours or days -- with heparin-bonded equipment, to permit evaluation of brain and heart, and, if indicated, for the heart to be repaired, assisted, or replaced. In cases of brain death, this procedure could serve organ donation.

V. ETHICS, PREDICTIONS

Every case of sudden coma or shock, with or without CA, deserves an all-out emergency resuscitation attempt (which is inexpensive), followed by life support long enough to predict outcome – at least 2 days after CA.[666-669] When to stop (expensive) prolonged life support is the greater challenge and dilemma. Ideally, CPCR should not be permitted to result in long-term survival with persistent severe brain damage (CPC 3 or 4). Cerebral resuscitation research should include studies on how to predict with certainty, as early as possible after CA, severe permanent brain damage (Table 2) with OPC 3 (consciousness but severely disabled) or CPC 4 (persistent vegetative state [PVS], permanent coma). "Good outcome" is CPC 1 or 2, with the survivor able to take care of himself. CPC 5 = OPC 5 = brain death. Early obstacles and confusions about determination and certification of brain death (which is only possible hours to days after restoration of normotension) organ donation, and discontinuance of controlled ventilation, have been overcome.[670,671]

In patients with PVS, discontinuance of all life support (including artificial airway, ventilation, feeding, and hydration; antibiotics; and emergency surgery) has been declared ethically justified.[670-676] Extraordinary means of life support in futile situations is considered unethical. The most challenging ethical dilemmas are CPC 3 and the gray zone between CPC 3 and 4, namely unresponsive patients in end-stage senile dementia (Alzheimer's disease). When CCM has nothing to offer, we consider prolonging undignified dying in the end-stages of life, which are painful to patients and surroundings, as unethical deviations of modern medicine.

PVS is cerebral (supratentorial) death (apallic syndrome), without destruction of the medulla, that is, with continued spontaneous breathing. This state can also be called "death."[672-676] Determination with 100% certainty of the irreversibility of PVS is not always

possible. To our knowledge, after CA and ROSC, fixed pupils or no purposeful response to stimuli for one week after CA (even as early as 3 days), in the absence of hypotension, hypothermia, CNS depressants, or relaxants has not been followed by any case of "good" cerebral recovery (CPC 1 or 2) in clinical correlation statistics. Even absent cranial nerve reflexes (corneal and/or carinal and/or gag reflex) as early as 12-24 h after CA, correlated with 100% poor outcome. Certain clinical and laboratory measurements (e.g., somatosensory evoked potentials; CSF enzyme levels) on day 3 after CA permit prognostication of PVS with near-certainty in the majority (but not all) cases of postcardiac arrest coma.

Cerebrospinal fluid (CSF) analysis might have adjunctive value. There were correlations of creatine-kinase BB and lactate dehydrogenase peaks in the CSF at 2-3 days after CA, in dogs[149] and patients,[677-681] with severity of insult and with poor neurologic outcome. Correlations with outcome of CBF and cerebral metabolism and/or NMR spectroscopic non-invasive evaluation of brain chemistry (e.g., energy charge) should be explored. In coma after TBI or FBI, reliable early prognostication is not possible. CSF analysis can reveal high enzyme levels from small, not-incapacitating lesions. Coma or stupor after TBI can last for months, due to edema or hemorrhages, and be followed by late awakening.

Decisions to "let die" are difficult in coma after CA and ROSC. The primary physician and intensivist, with input from other specialists, and in communication with the patient's proxies, should decide on the appropriate level of care: all-out life support; general medical care; general nursing care; or no extraordinary measures and compassionate terminal care.

Clinical resuscitation research is possible only with *waiving* of the prospective *informed consent* requirement for entering patients into studies. This must be done within seconds of recognition of the emergency. Waiving consent has proved to be feasible and acceptable.[682-685]

This must be legalized, provided the incremental (relative) risk is low and the study is approved by an institutional peer-review board. Randomized clinical trials are meant for evaluating novel methods to reverse death. These methods should have proven beneficial physiologic effects in clinically realistic animal models. This raises the ethical dilemma of randomly withholding a simple, safe, inexpensive treatment, such as mild hypothermia, which was proven to enhance the chance of survival without brain damage in clinically realistic animal models.

VI. CONCLUSIONS AND RECOMMENDATIONS

Since the 1970s, CPCR research has yielded much new information of scientific importance and documentation of several promising new therapies of clinical importance: hypertensive reperfusion after CA and ROSC; and mild hypothermia (induced as soon as possible and continued for at least 12 h). There has so far been no documented breakthrough effect of pharmacologic resuscitation potentials, probably because of the multifactorial complexity of the cerebral postresuscitation syndrome. Clinical feasibility and side-effect trials are needed in patients with sick hearts. The unreliability of outcome data in rodent models, the limitations of clinical trials, and the inadequate funding of reliable outcome models in high animal species have retarded implementation in patients of resuscitation potentials to save at least some "hearts and brains too good to die."[2,686]

For clinicians, we *recommend* the "CPCR system 2000" (Figure 6): (a) a brain orientation of standard CPR BLS-ALS-PLS (Table 3); (b) for cases resistant to external CPR-ALS, clinical feasibility and ROSC trials of improved external CPR methods and of an early switch to open-chest CPR or emergency CPB; (c) clinical feasibility and side-effect trials of the physical combination treatment of CBF promotion and mild hypothermia (the most effective cerebral resuscitation protocol yet documented in dogs and patients). This requires clinical feasibility trials of novel methods for rapid brain cooling (Table 4).

Therapeutic hypothermia – in general -- it is the only treatment from which some patients may benefit, even when mild cooling is induced late, as in the ICU. All cerebral resuscitation attempts after normothermic insults can be expected to be beneficial the earlier the cooling is initiated. The medical and lay public need clarification of differences: 1) between spontaneous-uncontrolled-accidental hypothermia and induced-controlled-therapeutic hypothermia; 2)

between cooling in the presence of circulation vs during CA; 3) between different temperature levels; and 4) between the following 10 emergency conditions for which different therapeutic hypothermia strategies might be beneficial.

Some benefit from various hypothermic strategies' has already been *documented* for the following indications: 1) Protection-preservation for elective or emergency surgery on heart, large vessels, or brain. 2) Resuscitation after normothermic CA (the main topic of this chapter). 3) Resuscitation from stroke (ischemic or hemorrhagic). 4) Resuscitation from traumatic brain injury. 5) Resuscitation from spinal cord injury.

Hypothermic strategies' benefit is *suspected* for at least five other conditions, which, however, are in need of more laboratory investigations before embarking on clinical trials: 6) Suspended animation for presently unresuscitable CA (as discussed above). 7) Traumatic hemorrhagic shock. 8) Sepsis and septic shock. 9) Myocardial infarction without CA, where mild hypothermia might reduce infarct size, but moderate hypothermia should be avoided as it can cause lethal arrhythmias. 10) Intractable status epilepticus (very rare).

Some of the greatest breakthroughs in medicine, such as anesthesia, antibiotics, insulin, and cortisone, saved many lives prior to any discovery of the molecular mechanisms of these treatments. Mechanism-oriented studies in rodents or in vitro, although scientifically important, are of only preliminary value, leading to the decisive outcome-oriented studies in whole organisms of animals high on the phylogenetic scale.

Cerebral resuscitation potentials to be researched further should be ranked according to their importance – 1) their scientific (theoretical, mechanism-oriented) importance; 2) their clinical importance for some cases; and 3) their socioeconomic importance for many humans.

Implementation should depend also on feasibility and affordability. A combination of these rankings might guide funding priorities.

The US Food and Drug Administration must recognize the limitations of randomized clinical outcome studies in resuscitation research; the limitation of studies in rats; and the importance of CPCR research with outcome models in large animals. Conditions causing sudden coma without CA should also be evaluated. The multifactorial pathogenesis of the postresuscitation syndrome calls for more than one agent, namely the need to evaluate combination treatments. How to explore and document outcome effects of combination treatments in a cost-effective manner is a challenge. The transfer to general patient care of novel CPCR methods found effective in large-animal outcome models should begin in community EMS systems with ongoing case registries that incorporate ongoing evaluation of all cases of sudden coma or shock.

One of us (PS) recommends that novel CPCR methods that are simple and inexpensive, and that significantly improved overall and cerebral outcome (without undesirable side effects) in two to three reliable reproducible large-animal outcome studies, should first be tested for safety and feasibility in clinical trials. If found safe, feasible, and economically possible for use in patients, such novel treatments should then be approved for general clinical use, without insisting on statistical "benefit" in expensive, time-consuming, randomized clinical trials, which are unreliable, uncontrollable, and often misleading. Pathophysiologic-therapeutic facts cannot be proved by epidemiologic correlation statistics. Randomized clinical trials of cerebral resuscitation cannot discriminate between the ability of a treatment to mitigate brain damage in selected cases and the absence of any treatment effect. Those who insist on such studies should at least increase the ability to reveal benefit in some cases, by excluding obviously hopeless

cases and immediately reversible arrests, and including only skilled, specially trained resuscitation teams and EMS systems.[2]

The goal of cerebral resuscitation research remains unchanged:[687,688] To help an increasing proportion of people stricken with an unexpected brain-damaging terminal state or clinical death, to return to full lives with healthy minds -- to restore "*mens sana in corpore sano*" (Decimus Iunius Juvenalis, Roman poet and satirist, about 100 A.D.).

ACKNOWLEDGEMENTS

Asmund and Tore Laerdal, Lyn Yaffe, M.D., and the U.S. Department of Defense enabled much of the research in Pittsburgh summarized in this chapter. We are grateful for input from Drs. N. Abramson, R. Basford, N. Bircher, L. Ernster, R. Hayes, P. Kochanek, L. Jenkins, A. Nozari, J.W. Severinghaus, B. Siesjo, F. Sterz, S. Tisherman, and X. Wu. Fran Mistrick, Valerie Sabo, and Brad Stezoski helped with preparation of the manuscript and images. Patricia Boyle helped with editing.

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TABLE 1

Glasgow Coma Score and Pittsburgh Brain Stem Scale for Early Postarrest Evaluation of Patients

Glasgow Coma Score (GCS)
Teasdale and Jennett: Lancet 2:81, 1974.

If patient is under the influence of anesthetics, sedatives, or neuromuscular blockers, give best estimate of each item. Write number in box to indicate status at time of this examination.

(A) *Eye Opening*

Spontaneous	= 4
To speech	= 3
To pain	= 2
None	= 1 ☐

(B) *Best Motor Response*
(extremities of best side)

Obeys	= 6
Localizes	= 5
Withdraws	= 4
Abnormal flexion	= 3
Extends	= 2
None	= 1 ☐

(C) *Best Verbal Response*
(if patient intubated, give best estimate)

Oriented	= 5
Confused conversation	= 4
Inappropriate words	= 3
Incomprehensible	
Sounds	= 2
None	= 1 ☐

Total GCS
(best GCS = 15)
(worst GCS = 3)

Pittsburgh Brain Stem Scale (PBSS)
Brain Resuscitation Clinical Trial (BRCT), 1980s.

Lash reflex present	yes = 2
(either side)	no = 1 ☐
Corneal reflex present	yes = 2
(either side)	no = 1 ☐
Doll's eye or ice water calorics reflex present	yes = 5
(either side)	no = 1 ☐
Right pupil reacts to light	yes = 2
	no = 1 ☐
Left pupil reacts to light	yes = 2
	no = 1 ☐
Gag, cough or carinal reflex present	yes = 2
	no = 1 ☐

Total PBSS
(best PBSS = 15)
(worst PBSS = 6)

Patient condition at time of examination:

Patient condition at time of examination:

Check all that apply: ☐ Anesthesia/heavy sedation.

☐ Paralysis (partial or complete neuromuscular blockade).

☐ Intubation. ☐ None of the above

TABLE 2

Overall and Cerebral Performance Categories for Outcome Evaluation of Patients

Cerebral Performance Category (CPC)

Evaluate only cerebral performance capabilities — Check one

CPC 1. *Good cerebral performance*
Conscious, alert, normal cerebral function. May have minor psychologic or neurologic deficits that do not significantly compromise cerebral or physical function. ☐

CPC 2. *Moderate cerebral disability*
Conscious, alert, normal cerebral function for activities of daily life (e.g., dress, travel by public transportation, food preparation). May have hemiplegia, seizures, ataxia, dysarthria, dysphasia, or permanent memory or mental changes. ☐

CPC 3. *Severe cerebral disability*
Conscious, has at least limited cognition. Dependent on others for daily support (i.e., institutionalized or at home with exceptional family effort) because of impaired brain function. Includes wide range of cerebral abnormalities, from ambulatory patients who have severe memory disturbance or dementia precluding independent existence to paralyzed patients who can only communicate with their eyes (e.g., the locked-in syndrome). ☐

CPC 4. *Coma/vegetative state*
Not conscious, unaware of surroundings, *no cognition*. No verbal or psychologic interaction with environment. May appear awake because of spontaneous eye opening or sleep-wake cycle. Includes all degrees of unresponsiveness that are neither CPC 3 (conscious) nor CPC 5 (coma that satisfies brain death criteria). ☐

CPC 5. *Brain death* (with beating heart) or *death* (without beating heart). Apnea, areflexia, "coma," EEG silence. ☐

CPC A. *Anesthesia* (CNS depressant)
Uncertain as to above categories because of anesthetic, other CNS depressant drug, or relaxant effects. ☐

Time achieved ☐ Hour ☐ Minute

Overall Performance Category (OPC)

Evaluate actual overall performance — Check one

OPC 1. *Good overall performance*
Conscious, alert, capable of normal life. Good cerebral performance (CPC 1). Generally fit or only minor noncerebral organ system dysfunction. ☐

OPC 2. *Moderate overall disability.*
Conscious, alert. Moderate cerebral disability alone (CPC 2) or moderate disability from noncerebral organ system dysfunction alone or both. Performs independent activities of daily life (dress, travel, food preparation) or able to work in part-time sheltered environment. Disabled for competitive work. ☐

OPC 3. *Severe overall disability*
Conscious. Severe cerebral disability alone (CPC 3) or severe disability from noncerebral organ system dysfunction alone or both. Dependent on others for daily support. ☐

OPC 4. *Coma/vegetative state*
Definition same as CPC 4.
With or without extracerebral organ dysfunction. ☐

OPC 5. *Death* (without beating heart)
Apnea, areflexia, "coma," no pulse. ☐

OPC A. *Anesthesia* (CNS depressant)
Uncertain as to above categories because of anesthetic, other CNS depressant drug, or relaxant effects. ☐

Time of determination ☐ Date ☐ Hour ☐ Minute

Compared with baseline status before the insult, the patient's *intellectual functions now* are (check one in each column):

	Patient opinion	Family opinion	Examiner opinion
Unchanged(1)	☐	☐	☐
Worsened (2)	☐	☐	☐
Unsure (3)	☐	☐	☐
Other or unable to Determine (4)	☐	☐	☐

Explain _____

Source: OPC -- Jennett and Bond, Lancet 1:480, 1975.
CPC and OPC – Safar and Bircher, CPCR, Saunders 1988.
Reflects cerebral *plus* noncerebral status

TABLE 3

Recommended Brain-Oriented Standard Basic-Advanced-Prolonged Life Support (BLS-ALS-PLS)

BLS-ALS

- Life supporting first aid (LSFA) skill acquisition by the lay public.
  - Vigorous CPR-BLS
  - steps A-B-C
- Start external exposure cooling of head and trunk
- Minimize arrest time with earliest automatic external defibrillation (AED), even by laypersons
- Increase perfusion pressure during external CPR with early (i.v. or intratracheal) epinephrine or other vasopressor
- Titrate i.v. vasopressor after ROSC
- Explore prolonged mechanical external CPR
- Give buffer in prolonged no-flow, low-flow
- In ALS-resistant cases, switch early to open-chest CPR or CPB

After ROSC, give brief hypertensive bout (systolic arterial pressure 150 to 200 mm Hg

- Use epinephrine, norepinephrine or dopamine
- Continue with controlled normotension or mild hypertension
  (titrated fluids dopamine, dobutamine, or other cardiovascular drugs)
- Control normoxia, normocarbia
- Aim for Tty 34°C as soon as possible during A-B-C or after ROSC (see Table 4)
- Check blood glucose level and keep it at 100-200 mg/dl
  (give glucose load IV if prearrest coma or seizures)

PLS

- Control normotension or mild hypertension, normoxia, normocarbia
- Restore blood volume
- Control base deficit at ± 5 mmoL/L
- Monitor brain temp. (Tty or Tnp) and core temp. (Tpa or Tcv or Tes)
- Maintain mild (34°C) resuscitative cerebral hypothermia from ROSC to 12-24 h
- Prevent or correct even mild hyperthermia
- Immobilize with softening doses of relaxant
- Sedate as needed to prevent shivering (titrated meperidine, diazepam, barbiturate)
- Control seizures
- Keep pupils small
- Conduct hemodynamic monitoring as feasible to guide administration of drugs and fluids

Keep hematocrit at 30% to 35%; electrolytes normal; plasma COP 15 mm Hg;

- serum osmolality 280-330 mosm/L
- Fluids i.v. (no dextrose in H_2O; give dextrose 5% or 10% in NaCl 0.25% or 0.5%, e.g., 50 ml/kg/24 hours)
- Maintain fluid balance; acid-base balance; alimentation
- Use standard intensive care life support, including head slightly elevated, turning trunk side to side

TABLE 4

Rapid Induction of Mild Resuscitative Cerebral Hypothermia in Comatose Patients After CA Clinical Cooling Methods

Ranked by rapid to low brain temperature	Ranked by feasibility to initiate Tty 34°C in patients with circulation
Intracarotid cold perfusion	*
Intra-aortic cold flush	*
Cardiopulmonary bypass with heat exchanger	*
Whole body ice-water immersion	impractical
Veno-venous extracorporeal blood shunt cooling	3
Peritoneal cold lavage	4
Esophagogastric, nasopharyngeal, i.v. cold infusion	2
Fanning or ice bags on skin	1

*Limited by vessel access time and availability of (portable) pump-heat exchanger.

LEGEND TO FIGURES

Figure 1. Diagram of different causes of cardiac arrest and their reversibility, with present standard normothermic CPR. Flow chart illustrates diagramatically the development of total circulatory arrest, as it happens *suddenly* (terminal states #1 as in VF or #2 as in a primary asystole); *over minutes* (terminal states #3-5); or *protracted* (terminal states #6-8). "Clinical death" is defined as "total circulatory arrest with potential reversibility to survival without brain damage.[11] Longest duration of clinical death that is reversible depends on terminal state, temperature, resuscitation methods, CPR (low-flow) time, and the post-resuscitation disease. After restoration of circulation, there are various possible outcomes. From Safar P: The Pathophysiology of Dying and Reanimation. In, Schwartz G, et al (eds): *Principles and Practice of Emergency Medicine*. Philadelphia, WB Saunders, 1985, pp 2-41.

Figure 2: American Heart Association algorithm for out-of-hospital management of patients with sudden cardiac death (from Guidelines 2000 for CPR-ECC, AHA; pp 1-67, ref. 6).

Figure 3: Diagram of post-cardiac arrest reperfusion failure in brain and extracerebral organs. Reperfusion failure in brain (proven) and extracerebral organs (suspected) after VF-CA and CPR (or CPB) for ROSC. After CA no-flow of 10-15 min, despite control of normal MAP, CBF and cardiac output go through four postarrest stages: Stage I, multifocal no-reflow (*), which can be prevented or overcome with high reperfusion pressures. Stage II, brief diffuse global hyperemia. Stage III, delayed protracted global hypoperfusion (**), accompanied by normal or super-normal global cerebral O_2 uptake between 2 h and 12-24 h (mismatching). Stage IV, normalization of CBF and $CMRO_2$ with awakening; or persistent coma with low CBF and $CMRO_2$.

Figure 4: *How cerebral neurons die after temporary ischemia.* Diagram of the very complex, partially hypothesized biochemical cascades in vital organ cells (e.g., cerebral neurons) during and after cardiac arrest. Normally, intracellular ($[Ca^{2+}]i$) to extracellular($[Ca^{2+}]i$) calcium gradient is 1:10,000 (i.e., 0.1 μmol: 1 mmol).

Calcium regulators include calcium/magnesium. ATPase, the endoplasmic reticulum (ER), mitochondria (M), and arachidonic acid (AA). With stimulation, different cell types respond with an increase in $[Ca^{2+}]i$, because of release of bound Ca^{2+} in the ER, and influx of $[Ca^{2+}]$, or both.

During sudden, complete ischemic anoxia (cardiac arrest) (left side), oxygen stores in the brain are consumed in about 15 seconds. The level of energy (phosphocreatine [PCr] and adenosine triphosphate [ATP]) decreases to near zero in all tissues at different rates, depending on stores of oxygen and substrate; it is fastest in the brain (about 5 min), and slower in the heart and other vital organs. This energy loss causes membrane pump failure, which causes a shift of sodium (Na^+) ions, water (H_2O) and (Ca^{2+}) from the extracellular into the intracellular space (cytosolic edema); and potassium (K^+) leakage from the intracellular into the extracellular space. Increase in $[Ca^{2+}]i$ activates phospholipase A_2, which breaks down membrane phospholipids (PL) into free fatty acids (FAA), particularly arachidonic acid (AA). Increase in $[Ca^{2+}]i$ also activates proteolytic enzymes, such as calpain, which may disrupt the cytoskeleton (CS) and possibly the nucleus. In mitochondria, hydrolysis of ATP to adenosine monophosphate (AMP) leads to an accumulation of hypoxanthine (HX). Increased $[Ca^{2+}]i$ may enhance conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO), priming the neuron for the production of the oxygen free radical (O_2^-), although this pathway is of questionable importance in neurons. X, xanthine; UA, uric acid. Excitatory amino acid neurotransmitters (EAA), particularly glutamate and aspartate, increase in extracellular fluid. Increased [EAA]*e* activates n-methyl-d-aspartate (NMDA) and non-NMDA receptors (R), thereby increasing calcium and sodium influx and mobilizing stores of $[Ca^{2+}]i$. Increased extracellular potassium activates EAA receptors by membrane depolarization.

Glycolysis during hypoxia results in anaerobic metabolism and lactic acidosis, until all glucose is used (in the brain, during anoxia after about 20 min). This lactic acidosis, plus inability to wash out CO_2, results in a mixed tissue acidosis that adversely influences neuronal viability. The net effect of acidosis on the cascades during and after ischemia is not clear. Mild acidosis may actually attenuate NMDA-mediated $[Ca^{2+}]i$

accumulation. Without reoxygenation, cells progress via first reversible, later irreversible structural damage to necrosis of all neurons or myocytes, homogeneously, at specific rates for different cell types.

During reperfusion and reoxygenation (right side), lactate and molecular breakdown products can create osmotic edema and rupture of organelles and mitochondria. Recovery of ATP and PCr and of the ionic membrane pump may be hampered by hypoperfusion as a result of vasospasm, cell sludging, adhesion of neutrophils (granulocytes) (N), and capillary compression by swollen astrocytes, which also help to protect neurons by absorbing extracellular potassium. Capillary (blood-brain barrier [BBB]) leakage results in interstitial (vasogenic) edema. Increased concentrations may be formed of at least four free radical species that break down membranes and collagen, worsen the microcirculation, and possibly also damage the nucleus. These species include superoxide (O_2-) leading to hydroxyl radical (•OH) (via the iron-catalyzed $Fe^{+++} \rightarrow Fe^{++}$, Haber-Weiss/Fenton reaction); free lipid radicals (FLR); and peroxynitrite ($OONO^-$). O_2^- may be formed from several sources: a) directly from AA metabolism by cyclooxygenase; b) by the previously described XO system; c) via quinone-mediated reactions within and outside the electron transport chain (from M); and d) by activation of NADPH-oxidase in accumulated neutrophils in the microvasculature or after diapedesis into tissue. Increased O_2^- leads to increased hydrogen peroxide (H_2O_2) production as a result of intracellular action of superoxide dismutase (SOD). H_2O_2 is controlled by intracellular catalase (c). Increased O_2- further leads to increased $^{\bullet}OH$ because of conversion of H_2O_2 to OH, via the Haber-Weiss/Fenton reaction, with iron liberated from mitochondria. This reaction is promoted by acidosis: OH and $OONO^-$ damage cellular lipids, proteins, and nucleic acids.

Also, AA increases activity of the cyclooxygenase pathways to produce prostaglandins (PGs), including thromboxane A_2, the lipoxygenase pathway to produce leukotrienes (LTs); and the cytochrome P-450 pathway. These products can act as neurotransmitters and signal transducers in neuron and glia, and can activate

thrombotic and inflammatory pathways in the microcirculation. Inflammatory reactions after ischemia have been proven to occur in extracerebral organs, focal brain ischemia, or brain trauma; but so far, they have not been proven after temporary complete global brain ischemia. Neuronal injury can signal interleukin-1 and other cytokines to be produced and trigger endogenous activation of microglia, with additional injury via QA, quinolinic acid, or other neurotoxins. In addition, tissue and/or endothelial injury--particularly associated with necrosis--can signal the endothelium to produce adhesion molecules (intracellular [ICAM], a-selectin, p-selectin), cytokines, chemokines, and other mediators, triggering local involvement of systemic inflammatory cells in an interaction between blood and damaged tissue.

Reoxygenation restores ATP through oxidative phosphorylation, which may result in massive uptake of $[Ca^{2+}]i$ into mitochondria, which are swollen from increased osmolality. Thus, mitochondria loaded with bound Ca^{2+} may self-destruct by rupturing and releasing free radicals. Increased $[Ca^{2+}]i$ by itself and by triggering free-radical reactions may result in lipid peroxidation, leaky membranes, and cell death. Neuronal damage can be caused, in part, by increased [EAA]*e* (excitotoxicity), resulting in increased $[Ca^{2+}]i$. During reperfusion, $[Ca^{2+}]i$ and increased [EAAs]*e* normalize. Their contribution to ultimate death of neurons is more likely through the cascades they have triggered during ischemia.

During ischemia and subsequent reperfusion, loading of cells and maldistribution of calcium in cells is believed to be the key trigger common to the development of cell death. This calcium loading signals a wide variety of pathologic processes. Proteases, lipases, and nucleases are activated, which may contribute to activation of genes or gene products (i.e., interleukin-converting enzyme, ICE or P53) critical to the development of programmed cell death (PCD, i.e., apoptosis); or inactivation of genes or gene products normally inhibiting this process. Activation of neuronal nitric oxide synthase (nNOS) by calcium can lead to production of NO, which can combine with superoxide to generate peroxynitrite ($OONO^-$). $OONO^-$ and $^{\bullet}OH$ both can lead to DNA injury and PCD, or protein and membrane peroxidation and necrosis, respectively. Nerve

growth factor (NGF), nuclear immediate early response genes (IERG) such as heat-shock protein, free radical scavengers (FRSs), adenosine, and other endogenous defenses (ED) work to lessen the damage.

There is substantial evidence that delayed cell death execution pathways are activated after ischemia, involving mitochondrial injury, cytochrome C release and caspase activation (right side of diagram). Neurotrophins, cytokines and other growth factors activate multiple receptor tyrosine kinases (RTK) signaling pathways linked to either pro-survival or pro-death activities. Several protein kinase cascades play a major survival role. The mitogen activated protein kinase (MAPK) cascade, involving multiple MAPKs, kinase RAF, MEK, and extracellular signal-regulated kinase (ERK), as well as phosphoinositide 3-kinase (PI3-K)/protein kinase B(PKB) pathways are best defined, but interactions involving phospholipase C gamma (PLCγ) protein kinase C (PKC) isoforms are also important. PKB mediates trophic signals via PI3-K and has numerous pro-survival actions. ERKs are also activated via neurotransmitter-linked protein kinase cascades involving small G protein-binding proteins (Ras) that activate Raf kinase and other MAPKs. ERKs have been implicated in both pro-survival and pro-death cascades. c-Jun NH2-terminal kinase (JNK) and other stress-activated protein kinases (SAPK) are also MAPKs that target similar nuclear transcription factors that modulate pro-death activity.

Figure 5: Improved cerebral and overall outcome after ventricular fibrillation (VF) cardiac arrest in reliable dog outcome models with immediate postarrest (resuscitative) mild cerebral hypothermia (34°C). Cooling was induced within 15 minutes of reflow. Each dot represents one experiment with 72 or 96 hours postarrest intensive care. Outcome as overall performance categories (OPCs).

Left: Control experiments after VF no flow 12.5 minutes and CPB achieved severe disability or coma (OPC 3 or 4) in 30 of 32 experiments. *Center*: Control experiments after VF no flow 10 minutes and external CPR achieved OPC 3 or 4 in 9 of 10 experiments. No controls achieved OPC 1 or OPC 5, documenting the models' reproducibility. Mild early postarrest hypothermia increased significantly the proportion of experiments with good outcome (OPC 1 or 2). *Right*: Data from the fifth mild hypothermia study of 1994,[67] as a model for clinical trials, as shown on the right and in open circles. (L = ref 63; W = ref 65; K = ref 66; S = ref 56,64; SP = ref 67).

Figure 6: The CPCR system of 2000 C.E. by Safar, modification of the ABC of 1961.[14] It consists of basic, advanced, and prolonged life support (BLS-ALS-PLS).

Fig. 1

Fig. 1

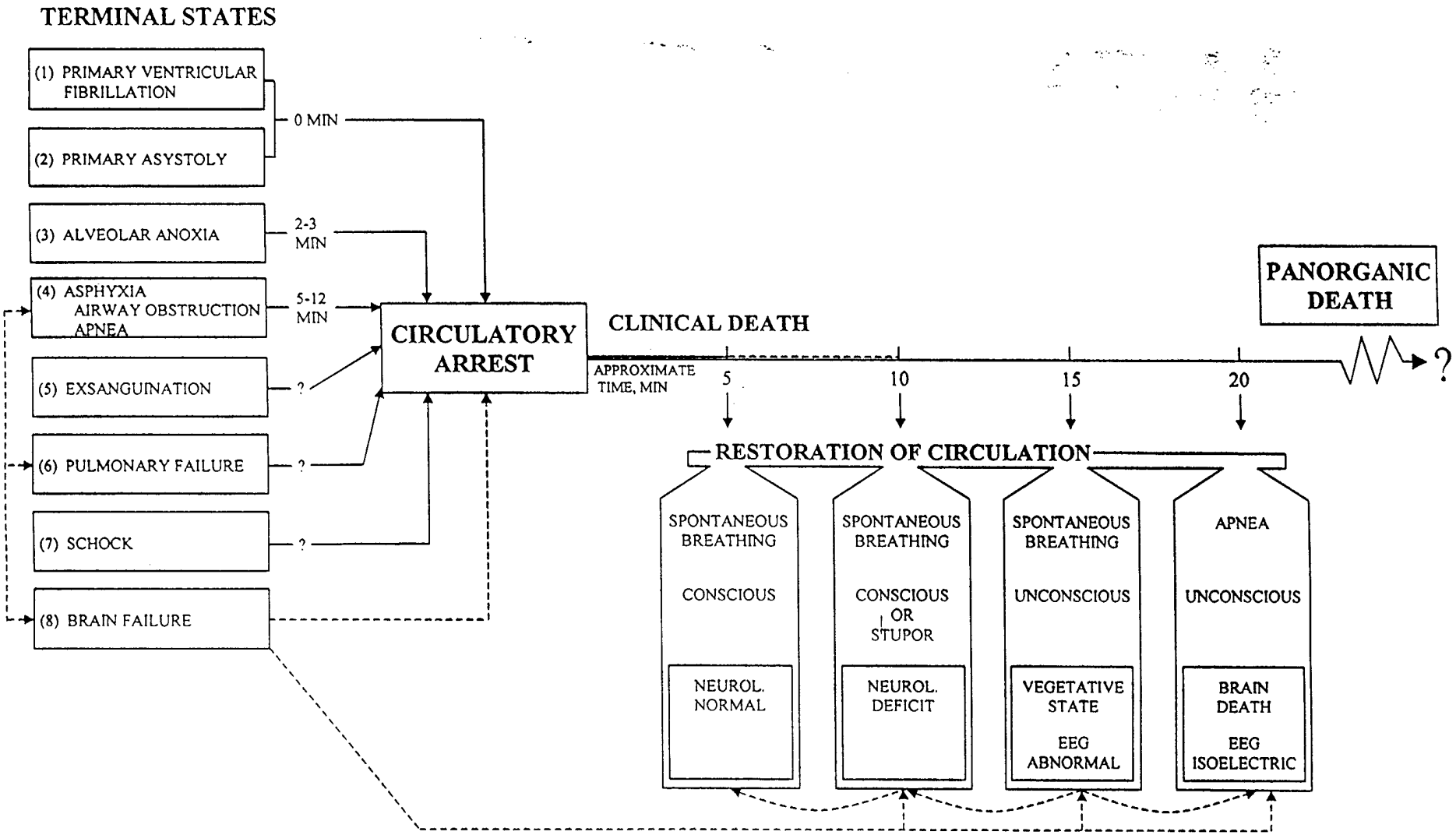
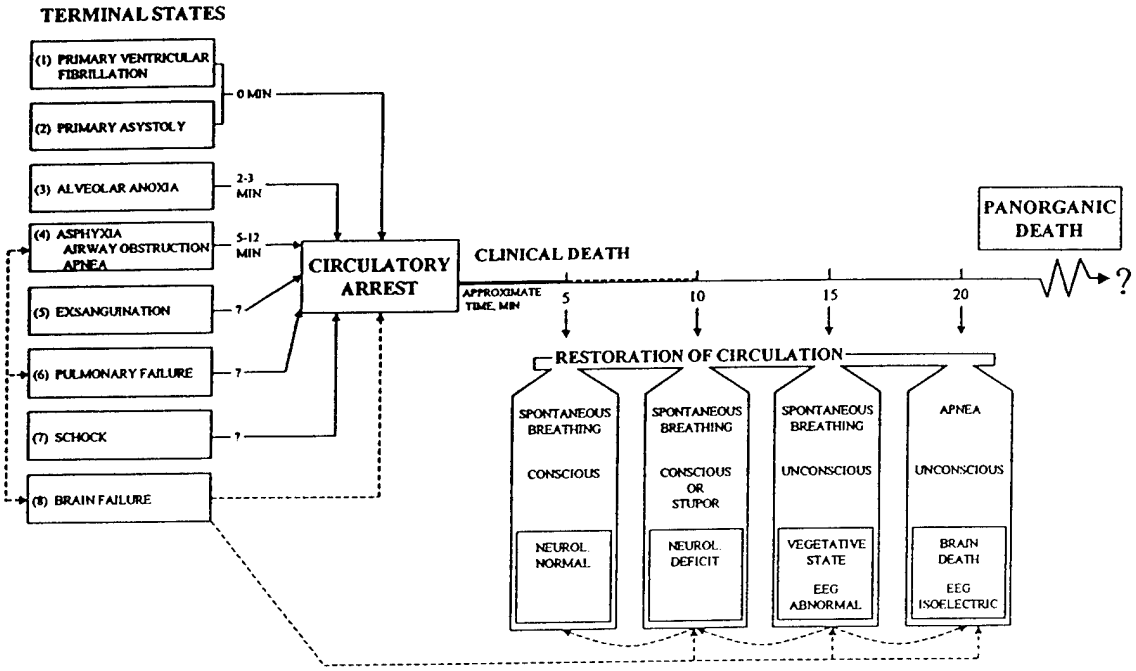

TERMINAL STATES
(1) PRIMARY VENTRICULAR FIBRILLATION
(2) PRIMARY ASYSTOLY
0 MIN
(3) ALVEOLAR ANOXIA
2-3 MIN
(4) ASPHYXIA
AIRWAY OBSTRUCTION
APNEA
5-12 MIN
(5) EXSANGUINATION
?
(6) PULMONARY FAILURE
?
(7) SCHOCK
?
(8) BRAIN FAILURE
CIRCULATORY ARREST
CLINICAL DEATH
PANORGANIC DEATH
?
APPROXIMATE TIME, MIN
5
10
15
20
RESTORATION OF CIRCULATION
SPONTANEOUS BREATHING
CONSCIOUS
NEUROL. NORMAL
SPONTANEOUS BREATHING
CONSCIOUS OR STUPOR
NEUROL. DEFICIT
SPONTANEOUS BREATHING
UNCONSCIOUS
VEGETATIVE STATE
EEG ABNORMAL
APNEA
UNCONSCIOUS
BRAIN DEATH
EEG ISOELECTRIC

Fig. 1


TERMINAL STATES
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CIRCULATORY ARREST
CLINICAL DEATH
APPROXIMATE TIME, MIN
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PANORGANIC DEATH
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RESTORATION OF CIRCULATION
SPONTANEOUS BREATHING
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BRAIN DEATH
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Fig. 2

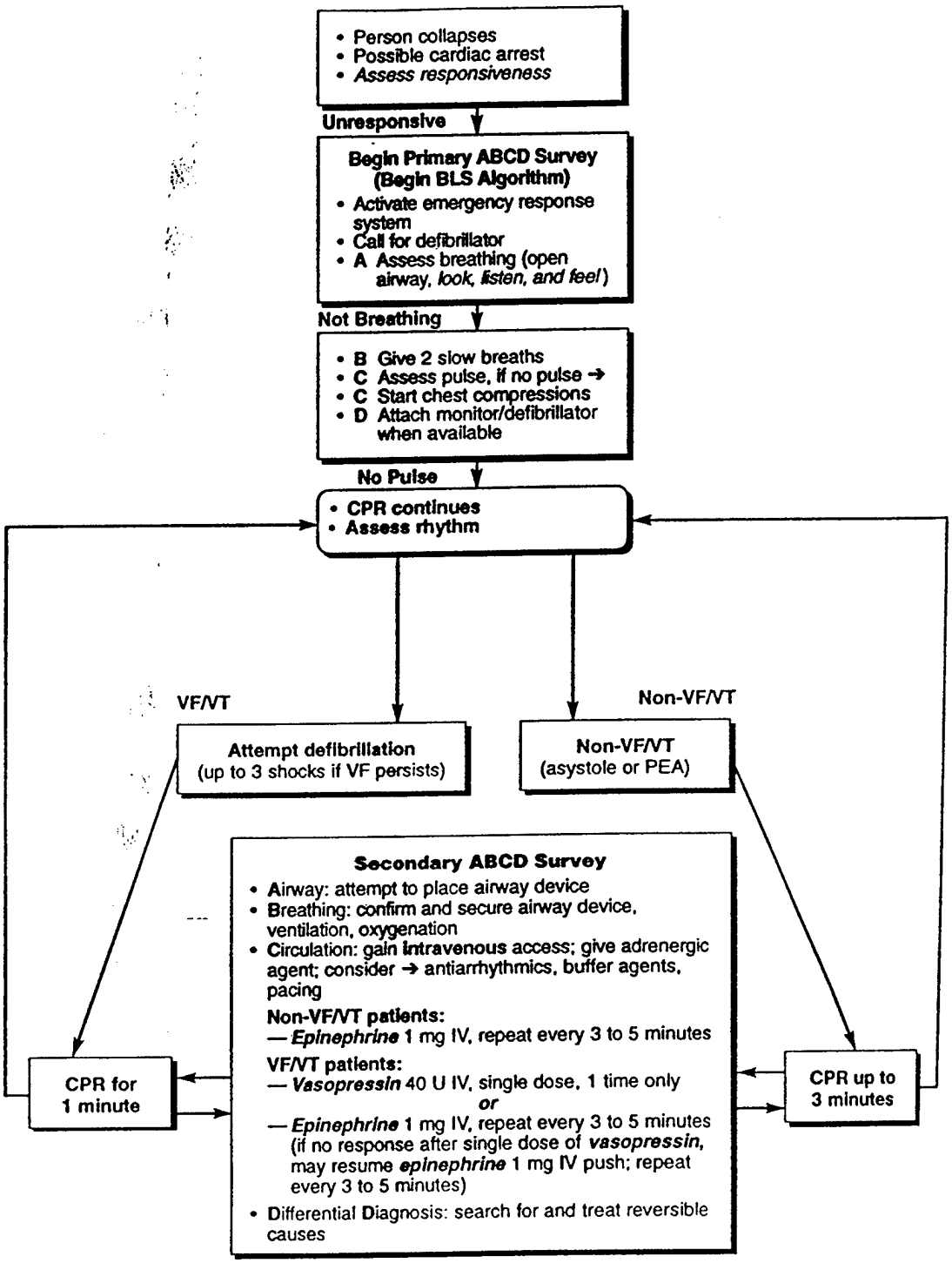

• Person collapses
• Possible cardiac arrest
• Assess responsiveness
Unresponsive
Begin Primary ABCD Survey
(Begin BLS Algorithm)
• Activate emergency response system
• Call for defibrillator
• A Assess breathing (open airway, look, listen, and feel)
Not Breathing
• B Give 2 slow breaths
• C Assess pulse, if no pulse →
• C Start chest compressions
• D Attach monitor/defibrillator when available
No Pulse
• CPR continues
• Assess rhythm
VF/VT
Attempt defibrillation
(up to 3 shocks if VF persists)
Non-VF/VT
Non-VF/VT
(asystole or PEA)
Secondary ABCD Survey
• Airway: attempt to place airway device
• Breathing: confirm and secure airway device, ventilation, oxygenation
• Circulation: gain intravenous access; give adrenergic agent; consider → antiarrhythmics, buffer agents, pacing
Non-VF/VT patients:
— Epinephrine 1 mg IV, repeat every 3 to 5 minutes
VF/VT patients:
— Vasopressin 40 U IV, single dose, 1 time only
or
— Epinephrine 1 mg IV, repeat every 3 to 5 minutes (if no response after single dose of vasopressin, may resume epinephrine 1 mg IV push; repeat every 3 to 5 minutes)
• Differential Diagnosis: search for and treat reversible causes
CPR for 1 minute
CPR up to 3 minutes

Fig. 3

Fig. 3

HYPOPERFUSION AFTER CARDIAC ARREST

Mismatching of O_2 delivery to O_2 uptake

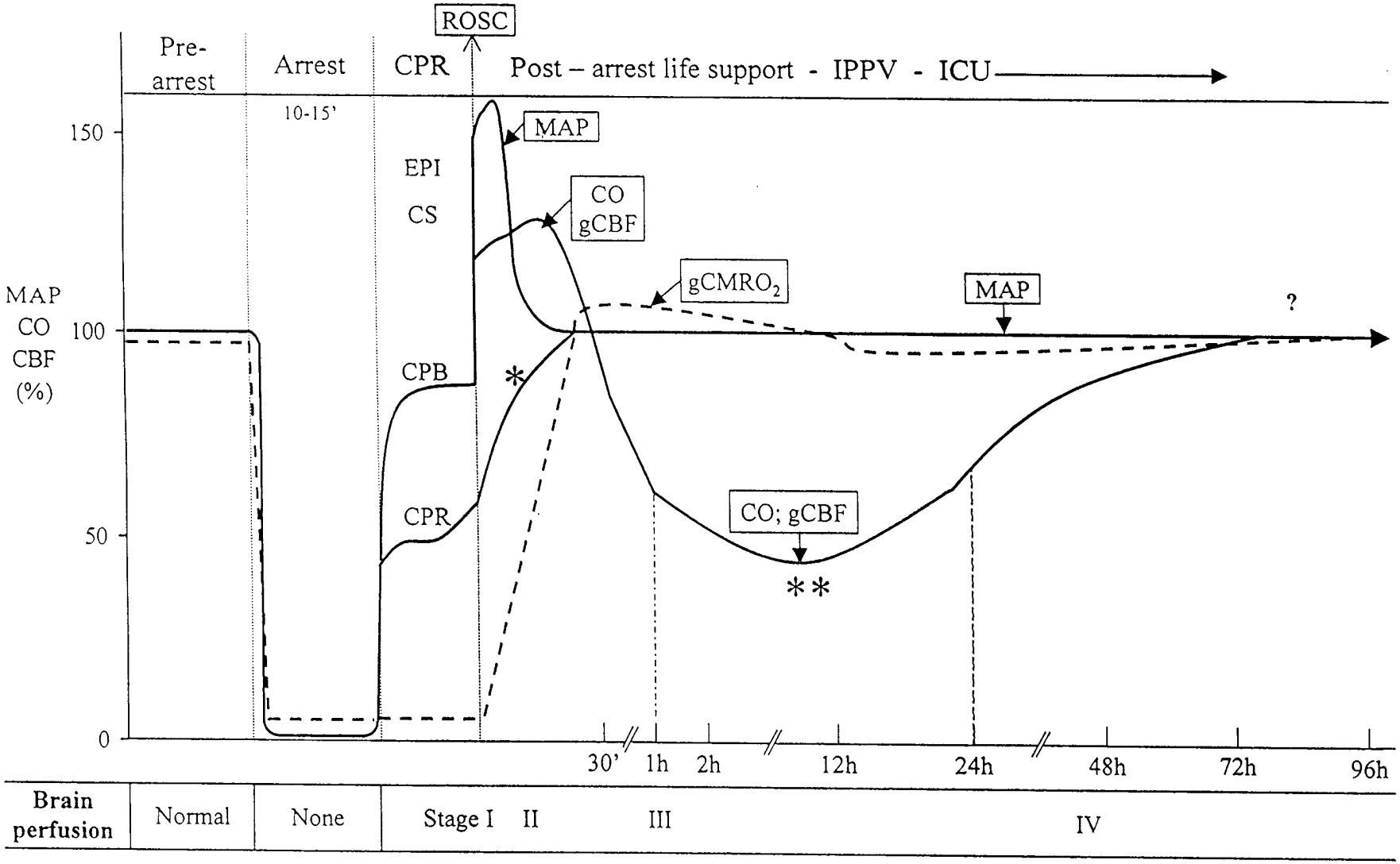


Fig. 3

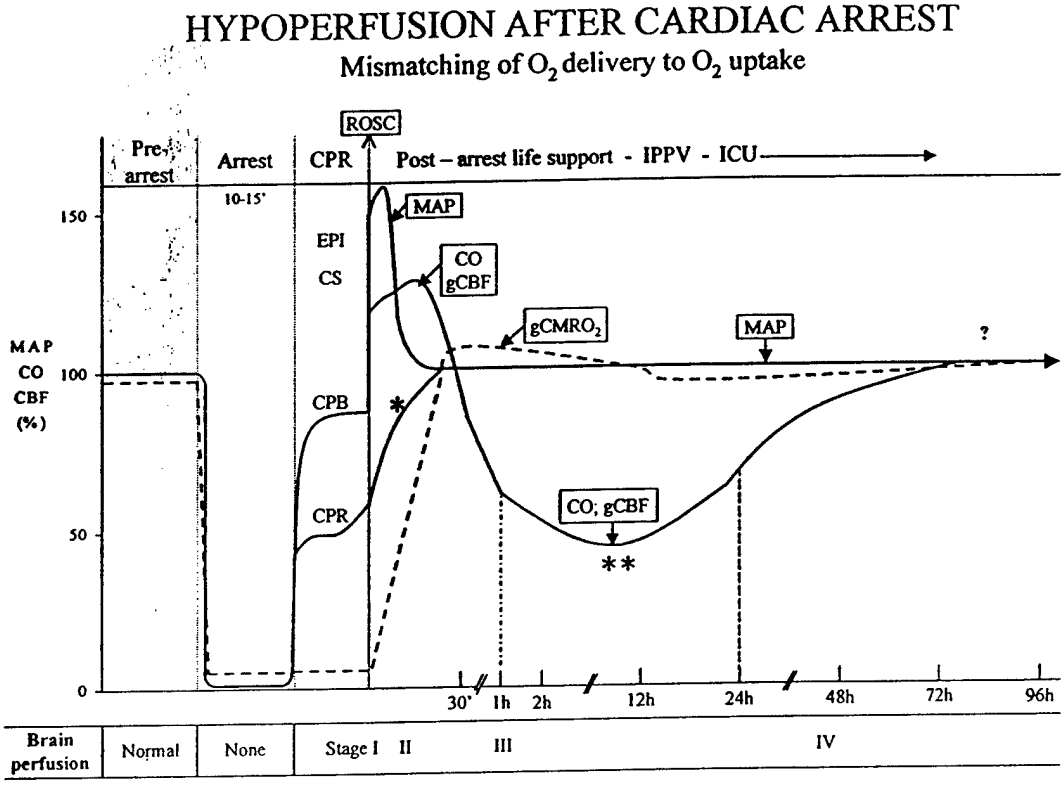

HYPOPERFUSION AFTER CARDIAC ARREST
Mismatching of O_2 delivery to O_2 uptake
Pre-arrest
Arrest
CPR
ROSC
Post – arrest life support - IPPV - ICU
10-15'
MAP
CO
gCBF
EPI
CS
gCMRO2
MAP
?
CPB
CPR
CO; gCBF
MAP CO CBF (%)
150
100
50
0
30'
1h
2h
12h
24h
48h
72h
96h
Brain perfusion
Normal
None
Stage I
II
III
IV

Fig. 4

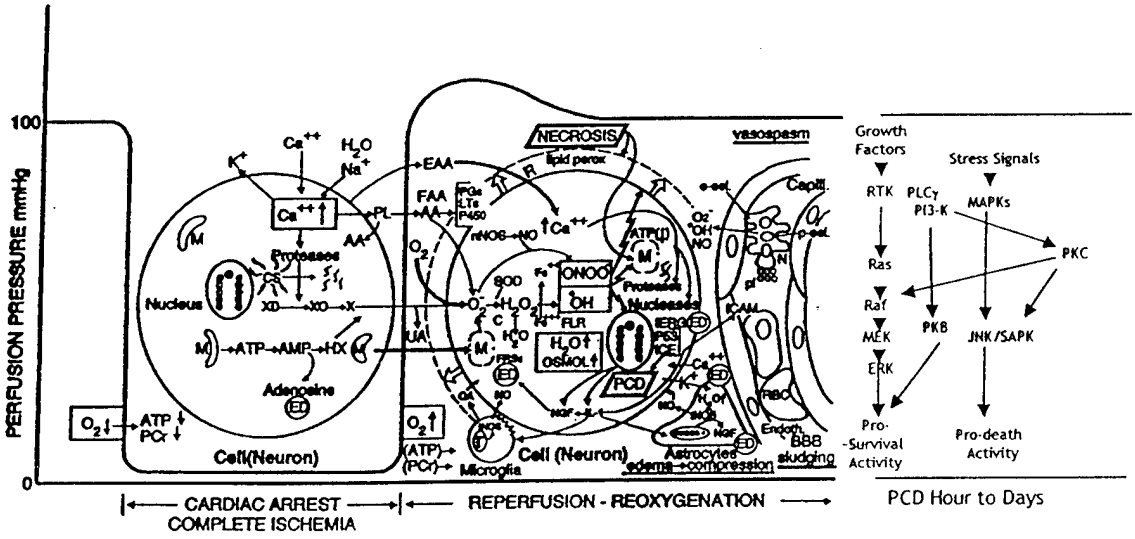

PERFUSION PRESSURE mmHg
100
0
CARDIAC ARREST
COMPLETE ISCHEMIA
REPERFUSION - REOXYGENATION
PCD Hour to Days
Cell(Neuron)
Cell (Neuron)
Microglia
Astrocytes
NECROSIS
vasospasm
Growth Factors
Stress Signals
RTK
PLCγ
PI3-K
MAPKs
PKC
Ras
Raf
MEK
ERK
PKB
JNK/SAPK
Pro-Survival Activity
Pro-death Activity

Fig. 4

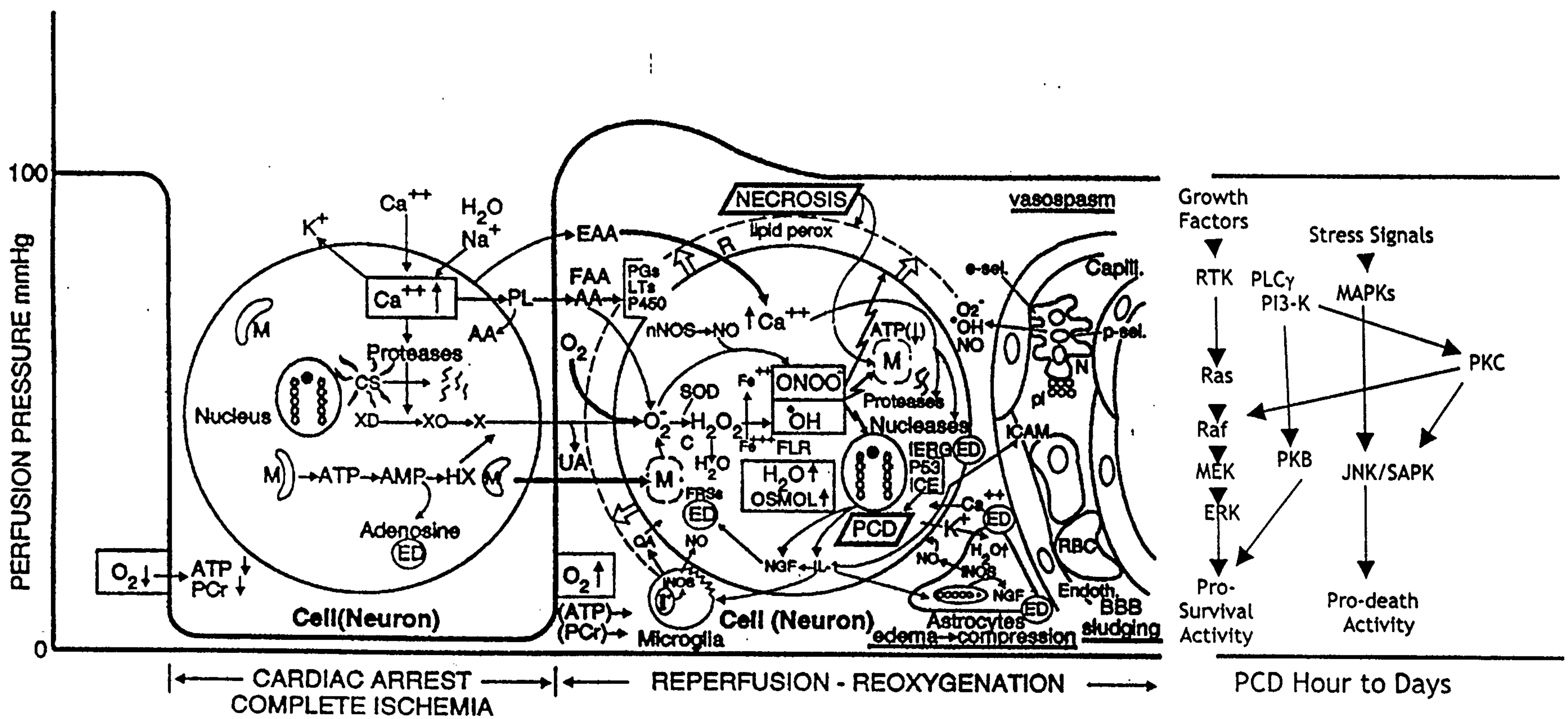

PERFUSION PRESSURE mmHg
100
0
CARDIAC ARREST
COMPLETE ISCHEMIA
REPERFUSION - REOXYGENATION
PCD Hour to Days
Cell(Neuron)
Cell (Neuron)
Nucleus
Proteases
Adenosine
NECROSIS
lipid perox
vasospasm
Capill.
e-sel.
p-sel.
ICAM
RBC
Endoth.
BBB
sludging
Microglia
Astrocytes
edema→compression
Nucleases
PCD
FLR
NGF
Growth
Factors
RTK
Ras
Raf
MEK
ERK
Pro-
Survival
Activity
PLCγ
PI3-K
PKB
Stress Signals
MAPKs
JNK/SAPK
Pro-death
Activity
PKC

Fig. 4

Fig. 5

RESUSCITATIVE MILD CEREBRAL HYPOTHERMIA IN DOGS
OUTCOME AFTER CARDIAC ARREST

	VF No-Flow 12.5 min -- CPB							VF No-Flow 10 min -- CPR			VF No-Flow 11 min	
Best OPC 24 - 96 h	Controls Tcv 37.5°C				Hypothermia Tcv 34°C during VF or reperfusion			Controls Tcv 37.5°C	Hypothermia Tcv 34°C after ROSC	Hypothermia Tcv 34°C during CPR	Controls Tcv 37.5°C	Hypothermia Tcv 34°C plus CBF Promotion
5 BRAIN DEATH DEATH												
4 COMA, PVS	•• • •	• • ••	• ••				•	• •			o o o	
3 SEVERE DISABILITY	• ••• ••	•	••	• • •	•	• • •	••	• • •	• • •	• •	o	o
2 MODERATE DISABILITY	••				• •	• • •	• • •	•	• • •	••		o
1 NORMAL					• • •	•			••	• •		o o o
p-values vs controls					<0.01	<0.05	<0.05		<0.01	<0.005		<0.01
References	L	W	K	S	L	W	K	S	S	S	SP	

Fig. 6

Cardio-Pulmonary-Cerebral Resuscitation (CPCR) 2000

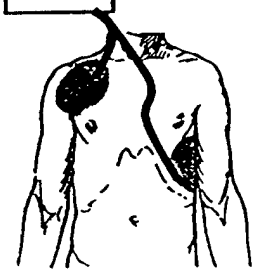
For Sudden Coma or Shock
!Act rapidly — seconds count!

BLS = **Life Supporting First Aid (LSFA)**

Basic Life Support

- IF UNCONSCIOUS – **TILT**
  - **A** – *AIRWAY CONTROL*
    tilt head back (+ jaw thrust + open mouth)
    if foreign matter
- IF NOT BREATHING – **BLOW**
  - **B** – *BREATHING CONTROL*
    mouth-to-mouth (nose)
- IF STILL NOT BREATHING AND LOOKS DEAD – **PUMP**
  - **C** – *CIRCULATION CONTROL*
    compress breastbone, about 2x/sec.
  - **D** – *DEFIBRILLATE* – **ZAP**
    call for and apply AED
    follow voice instructions
  - **E** – *EXPOSE – COOL IS "COOL"*
    EXTERNAL BLEEDING – Compress

AED



Professionals – Continue A-B-C-D

ALS
Advanced Life Support (titrated)

- i.v. Drugs – epi,norepi, other
  brief hypertension → normotension
- Intubate trachea
  bag-valve IPPV → mechanical
- Mild hypothermia ASAP
  ear temperature 34°C
- Fluids i.v.

PLS
Prolonged Life Support
ICU

Brain-oriented intensive care
normotension, normoxia, normocapnia
mild hypothermia (34°C) to 12 h
fluid, acid base, ect.

If coma after cardiac arrest - -
max. effort for at least 3 days

J Trauma
Submitted

Suspended Animation Can Allow Survival Without Brain Damage After Traumatic Exsanguination Cardiac Arrest Of 60 Min In Dogs

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Running Title: Suspended Animation and Traumatic Exsanguination

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Acknowledgements:

Franklin A. Bontempo, MD made valuable suggestions. Brian Slater, Sherman Culver, Alan Abraham and Murugan Subramanian helped with intensive care life support. This study was supported by grant DAMD 17-01-2-0038 of the US Army MRMC/TATRC and the Laerdal Foundation for Acute Medicine.

Presented at the American Society of Anesthesiologists' annual meeting, October 16, 2002, Orlando, Florida.

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Disclosure Statement

The following authors of this manuscript, entitled, "Suspended Animation Can Allow Survival Without Brain Damage After Traumatic Exsanguination Cardiac Arrest of 60 Min in Dogs," from the University of Pittsburgh, have no financial or proprietary interest in the subject matter or materials discussed in the manuscript. This includes (but is not limited to) employment, consultancies, stock ownership, honoraria, and paid expert testimony.

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ABSTRACT

Background: We have previously shown in dogs that exsanguination cardiac arrest of up to 120 min without trauma under profound hypothermia induced by aortic flush (suspended animation) can be survived without neurologic deficit. In the present study the effects of trauma are explored. This study is designed to better mimic the clinical scenario of an exsanguinating trauma victim, for whom suspended animation may buy time for resuscitative surgery and delayed resuscitation.

Methods: Fourteen dogs were exsanguinated over 5 min to cardiac arrest. Flush of saline at 2°C into the femoral artery was initiated at cardiac arrest 2 min and continued until a tympanic temperature (Tty) of 10°C was achieved. The dogs were then randomized into a control group without trauma (n=6) or a trauma group (n=8) which underwent, at start of cardiac arrest, spleen transection and left thoracotomy. During cardiac arrest, splenectomy was performed. After cardiac arrest of 60 min no flow, reperfusion with cardiopulmonary bypass was followed by intensive care to 72 h.

Results: All 14 dogs survived to 72 h with histologically normal brains. All control dogs were functionally neurologically intact. Four of 8 trauma dogs were also functionally normal. Four had neurologic deficits, although 3 required prolonged mechanical ventilation because of airway edema and evidence of multiple organ failure. Blood loss from the chest and abdomen was variable and was associated with poor functional outcomes.

Conclusions: Rapid induction of profound hypothermic suspended animation (Tty 10°C) can enable survival without brain damage after exsanguination cardiac arrest of 60 min no flow even in the presence of trauma. This technique may allow survival of exsanguinated trauma victims who now have almost no chance of survival.

INTRODUCTION

Despite advances in resuscitation techniques and in surgical management of trauma victims, survival rates remain extremely low in trauma patients who exsanguinate to cardiac arrest [1,2]. Emergency department thoracotomy to treat cardiac tamponade, control intra-thoracic hemorrhage, perform open-chest cardiac massage, and cross-clamp the aorta to optimize cerebral and myocardial perfusion and decrease intra-abdominal hemorrhage is often performed but the surgical team's race against the clock to achieve hemostasis is rarely successful, even when the underlying injury is technically repairable. Most patients die or suffer severe brain injury because these extraordinary efforts are not adequate to restore blood flow before the limit of tolerance under normothermia of 5 min circulatory arrest for the brain [3,4] and about 20 min for the heart [4,5].

In 1984, Bellamy and Safar considered these issues when reviewing data from the Vietnam War. It was clear that a new approach to resuscitation is needed [2]. Suspended animation, i.e., rapid induction of pharmacologic-hypothermic preservation, was introduced as a new concept for attempting resuscitation from cardiac arrest in presently unresuscitable victims [2,6]. The viability of brain and organism is preserved with suspended animation during cardiac arrest, to buy time for transport and resuscitative surgery, until restoration of spontaneous circulation or prolonged artificial circulation is possible. Using dog outcome models of exsanguination cardiac arrest, the Pittsburgh group has systematically explored suspended animation potentials. The initial studies included pressure-controlled hemorrhagic shock, rapid cooling via cardiopulmonary bypass (CPB), 60-120 min deep (15°C) or profound (<10°C) circulatory arrest, and resuscitation via CPB [7,8]. Since CPB can not be initiated rapidly enough, we have more recently explored induction of suspended animation via a rapid flush of ice-cold saline into the aorta. Hypothermic preservation induced within 5 min of circulatory arrest

through aortic or femoral cold saline flush has allowed long-term survival without brain damage after up to 120 min of no-flow cardiac arrest[9-12].

The experimental model in these studies involved exsanguination, but not major tissue injury. However, the majority of patients who experience hemorrhage severe enough to cause exsanguination cardiac arrest have a major vascular or solid organ injury with significant tissue trauma. The potential efficacy of suspended animation in traumatic exsanguination cardiac arrest has therefore been questioned, as trauma may affect the distribution of preservative cold flush, cause coagulopathy, and elicit inflammatory and other deleterious responses. In the present study, we aimed to determine the outcome after suspended animation in a clinically realistic dog model of exsanguination cardiac arrest with abdominal injury and thoracotomy. We hypothesized that additional trauma would worsen the chance of intact survival.

METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and the Department of Defense and was conducted in accordance with the Animal Welfare Act and other Federal statutes and national guidelines for the treatment of animals. All surgical procedures were performed by the same team in a designated veterinary surgical suite with sterilized instruments and aseptic procedures.

A total of 35 custom-bred hunting dogs (21 - 28 kg body weight, age 8 - 12 months) were used; 16 were used for pilot experiments to determine the trauma, hemorrhage and flush, 5 were used as blood donors, and 14 were used for the definitive study with exsanguination cardiac arrest of 60 min with or without laparotomy, splenic injury, and thoracotomy. Whole blood of donor dogs was collected into 1000 mL bags with 50 mL sodium citrate through a 19 Fr, right external jugular vein catheter, inserted under general anesthesia, and was stored at 6 °C for up to 7 days.

Preparation

The dogs were fasted overnight with free access to water. After sedation with ketamine (10 mg/kg i.m.), anesthesia was induced with halothane (2-4%) in N_2O/O_2 (50/50%) via a cone mask. The dogs were then intubated and mechanically ventilated (Harvard Piston Ventilator model 613, Harvard Apparatus, South Natick, MA) with a tidal volume of 15 mL/kg and the rate adjusted to maintain $PaCO_2$ 35 - 40 mmHg. A positive end-expiratory pressure of 5 cm H_2O was applied. Anesthesia was maintained during preparation with halothane 0.5 - 1.5% in N_2O/O_2 (50/50%) without neuromuscular blockade.

Temperature probes were inserted for measuring tympanic membrane (Tty), esophageal (Tes) and rectal temperatures (Tr). Tty was controlled at 37.5 ± 0.1 °C with heating blankets and heating lamps before the insult. Gastric and bladder catheters were inserted. Dextrose 5% in 0.45% sodium chloride was administered at 5 mL/kg/h via a peripheral i.v. cannula (18 gauge). A 10 Fr catheter was inserted into the left femoral artery for monitoring arterial blood pressure and blood sampling. A pulmonary artery catheter (7.5 Fr, Intellicath Continuous Cardiac Output Thermodilution Catheter, Baxter Co., Irvine, CA) was inserted via the left femoral vein and advanced into wedge position for pressure and temperature monitoring (Tpa), cardiac output determination and blood sampling. Arterial and central venous pressures and electrocardiogram were continuously recorded on a polygraph (Grass Model 7D Polygraph, Quincy, MA). Pulmonary artery pressure, pulmonary artery occlusion pressure, cardiac output, arterial and mixed venous blood gases, hemoglobin, hematocrit, serum sodium, potassium, glucose, and lactate levels were measured at regular intervals. Coagulation abnormalities were assessed using thromboelastography (TEG) (Thromboelastograph, Haemoscope Co., Morton Grove, IL). Reaction time (r), clot formation time (K), alpha angle and maximum amplitude (MA) were measured and recorded for each animal at baseline, and at 1, 3, 6, 9, 15, 24 and 72 h after reperfusion, using celite-activated whole blood at 37°C.

A 7-8 gauge cannula was inserted 3 cm into the right femoral artery for arterial cold flush after induction of circulatory arrest and, after cardiac arrest of 60 min, to return arterialized blood to the animal from the CPB circuit (CPB arterial cannula)[13,14]. The right external jugular vein was cannulated with a multiple-holed 19 Fr catheter, which was advanced to the level of the right atrium, for venous bleeding during exsanguination and later, for venous return to the CPB system.

Insult

In the trauma group, a midline laparotomy was performed providing exposure for the splenic injury. The abdomen was temporarily closed with towel clips.

In both groups, after two baseline measurements, halothane and intravenous fluids were discontinued, heating devices were turned off and the dogs were weaned to spontaneous breathing of N_2O/O_2 (70/30%) via a T-tube. When the canthal reflex returned, hemorrhage was initiated. Over a 5 min period the dogs were bled via the jugular venous cannula, and the blood was collected in 1000 mL bags with 50 mL sodium citrate for later reinfusion. The hemorrhage was controlled to achieve mean arterial pressure (MAP) of 40 mmHg at 2 min, 30 mmHg at 3 min, and 20 mmHg at 4 min, at which time, in the trauma group, the abdomen was re-opened and a standardized complete transection of the spleen was performed at its midpoint. At 5 min, to assure zero blood flow, ventricular fibrillation (VF) was induced with a 95 volts AC, 60 Hz transthoracic shock through subcutaneous needles for 2 sec, repeated as needed. Cardiac arrest was defined by identification of VF on the EKG and the loss of arterial pulsation. Total arrest time (no-flow) was 60 min.

Preservation and surgical hemostasis

After two min of no-flow (cardiac arrest), normal saline at 2°C was flushed in both groups through the CPB arterial cannula into the femoral artery at a rate of 1.7 L/min, using a roller pump, until Tty reached 10°C. In the trauma group, at 2 min no-flow, a left lateral thoracotomy was performed at the sixth intercostal space, exposing the intra-thoracic organs. At cardiac arrest 20 min, simulating the time needed for transport, splenectomy was performed. The

abdominal wall and the thoracotomy incision were, however, left open to detect possible bleeding after initiation of CPB.

Resuscitation

Reperfusion after cardiac arrest no-flow of 60 min was achieved in both groups with CPB [13,14], using heparin-coated circuits to avoid systemic heparinization (Medtronic – Carmeda bonded circuits, Grand Rapids, MI). The CPB system was primed with 400 mL of lactated Ringer's solution with 2 mEq/kg sodium bicarbonate. The flow was adjusted with a centrifugal pump (Biomedicus, Eden Prairie, MN), at 100 mL/kg/min. Reinfusion of the shed blood was titrated, aiming to maintain a MAP of 90 to 150 mmHg, and a central venous pressure of 10-15 mmHg. If necessary, epinephrine boluses of 5 μg/kg were administered and norepinephrine infusion was titrated to maintain the MAP within the targeted range. Gas flow through the CPB oxygenator was adjusted to keep $PaCO_2$ at 30-35 mmHg. The temperature of the water bath of the CPB heat exchanger was set to 5°C above Tty, until Tty reached 34°C. Controlled ventilation was resumed with 100% O_2, at a rate of 8 - 10 inspirations/min. The i.v. maintenance fluid was restarted with a flow of 100 mL/h. A base deficit of >6.0 mEq/L was treated with sodium bicarbonate.

When Tpa reached 32°C, defibrillation attempts were initiated with external DC counter-shocks of 150 J, increased by 50 J for repeated shocks. If spontaneous circulation was restored, the CPB flow rate was reduced to 75 mL/kg/min at 60 min, to 50 mL/kg/min at 90 min, and was stopped at 120 min. Bleeding into the abdomen or chest was controlled with ligation of involved vessels and with electrocautery. The abdominal and thoracic incisions were closed in layers, with a left-sided chest tube (28 Fr) inserted through the 7^{th} intercostal space along the midaxillary

line. Donor blood was transfused, if necessary, to maintain hematocrit above 25%. Partial cross-match was accomplished before all transfusions by adding a drop of the donor blood to the recipient dog's blood at room temperature and observing for macroscopic agglutination.

Intensive Care

After weaning from CPB at 2 h, controlled ventilation and circulatory support was continued to at least 20 h. Neuromuscular blockade was maintained with intermittent doses of pancuronium bromide (0.1 mg/kg i.v.). Sedation and analgesia were provided with N_2O/O_2 (50/50%) plus i.v. boluses of morphine (0.1-0.3 mg/kg), and diazepam (0.1-0.2 mg/kg) to prevent signs of wakefulness, e.g., mydriasis. Severe hypertension (MAP >150 mmHg) despite adequate analgesia was controlled with i.v. boluses of labetalol (0.25 - 0.5 mg/kg) or hydralazine (0.1 - 0.2 mg/kg).

Hypotension (MAP <90 mmHg) was treated with normalization of filling pressures by fluid administration (blood or 5% albumin depending on the hematocrit value) and with titrated norepinephrine. Standard intensive care included airway suctioning, periodic deep lung inflations, and position change (rotation). The dogs received Cefazolin (250 mg i.v.) every 8 h for infection prophylaxis.

At 20 - 24 h, paralysis was reversed with neostigmine (50 μg/kg) plus atropine (25 μg/kg) and the dogs were weaned to spontaneous breathing via T-tube. The chest tube was removed in the trauma dogs after >30 min of spontaneous breathing if signs of air leaks or ongoing blood loss were absent and if PaO_2 was maintained >100 mmHg on air and $PaCO_2$ was 30-40 mmHg. The dogs were extubated when they met the above-mentioned criteria and after their upper airway reflexes had returned. If the dogs could not be weaned to spontaneous breathing or

required continued circulatory support they were kept ventilated for an additional 24 h before new attempts at weaning. After extubation, the catheters were removed under brief N_2O - halothane anesthesia by cone mask. The dogs were transferred to a step-down unit to 72 h, with O_2 by mask and continuous monitoring of pulse rate and arterial O_2 saturation. Suspected pain was controlled with titrated i.v. doses of morphine (0.1-0.2 mg/kg), distress with i.v. diazepam (0.1 - 0.3 mg/kg). The maintenance fluid was dextrose 5% in NaCl 0.45% until 24 h, and dextrose 10% in NaCl 0.45% thereafter, until the dog was able to eat and drink. The dogs were continually monitored by technicians, with critical care physicians immediately available.

Outcome evaluation

Performance was evaluated according to overall performance categories (OPC 1 = normal; 2 = moderate disability; 3 = severe disability; 4 = coma; and 5 = death) [14]. Neurologic function was evaluated as neurologic deficit scores (NDS 0 - 10% = normal; 100% = brain death) [3,14]. OPC and NDS were evaluated every 8 h after extubation. Attempts were made to discontinue any sedation at least 4 h prior to final evaluations. If necessary, sedation was reversed with naloxone hydrochloride (narcotic antagonist) 1.5-6.0 μg/kg or with flumazenil (benzodiazepine antagonist) 0.1 mg i.v., repeated if needed.

After final outcome evaluation, for morphologic studies, the dogs were re-anesthetized with ketamine 10 mg/kg intramuscularly, followed by halothane 0.5 to 1.5% in N_2O/O_2 (50/50%). A left thoracotomy was performed, and the proximal descending aorta was ligated. A large-bore cannula was inserted proximal to the ligature. The dogs were then euthanized by infusing paraformaldehyde (4%, pH 7.4) into the aortic arch using a roller pump at a pressure of approximately 100 mmHg, with the right atrium opened, until clear fluid returned. A complete

necropsy was performed with scoring of macroscopic damage to extracerebral organs (minimal, mild, moderate or severe), taking into account the pattern, appearance and anatomic distribution of the lesions. One hour after perfusion fixation, the brain was removed. After cutting 3 mm thick slices, the same six slices of each brain were paraffin-embedded, cut into sections 4 microns thick, and stained with hematoxylin-eosin-phloxine. Using light microscopy, the same pathologist, blinded for treatment assignments, scored 19 distinct anatomic brain regions for severity and extent of ischemic neuronal changes, infarcts, and edema, as described previously [3]. The total brain histologic damage score (HDS) was the sum of all area scores. An HDS of > 40 represents moderate damage, and > 100 represents severe damage.

Statistical analysis

Data are presented as mean and standard deviation (SD) unless otherwise stated. Repeated measures analyses of variance were performed followed by Bonferroni/Dunn post-hoc tests to identify differences in hemodynamic parameters and temperature data between groups over time. NDS and HDS scores were analyzed using Mann-Whitney U Test, and Fisher's exact test was used to assess differences in OPC proportions (OPC 1 and 2 good outcome versus OPC 3,4 or 5 bad outcome) between groups. Pearson correlation coefficient was computed between the OPC and the volume of transfused blood, followed by Fisher's r to z transformation of the correlation coefficient to calculate a probability level. A p-value <0.05 was considered statistically significant.

RESULTS

Pilot experiments

Suspended animation induced by direct aortic (cannulation of the descending thoracic aorta through a left thoracotomy) flush of cold saline in a model of traumatic (laparotomy, liver or spleen trauma) exsanguination cardiac arrest no-flow of 90 min consistently resulted in severe coagulopathy with flat TEG curves and rapid exsanguination from the vascular or soft tissue injuries, or multiple organ dysfunction (cardiovascular failure, respiratory failure, renal failure and neurologic failure [15,16]). In other pilot experiments with abdominal and thoracic trauma, the flush was initially cephalad through a balloon catheter (8 French, Cardeon Co.) placed in the mid-thoracic aorta via the femoral artery until the target Tty reached 10°C, and then in a caudad direction by deflating the balloon and compressing the proximal aorta manually (via thoracotomy). After 90 min cardiac arrest with trauma, all dogs died within 24 h of irreversible shock. In contrast, in experiments without trauma and the same exsanguination insult, 90 min no-flow cardiac arrest, and resuscitation, aortic flush via a catheter in the iliac artery resulted in good outcome[12]. With resuscitation from traumatic exsanguination cardiac arrest of 90 min not yet feasible in our model, an arrest duration of 60 min was chosen for the definitive study.

Resuscitation

All 14 dogs in the final series (both groups) were successfully resuscitated and survived to 72 h. Restoration of spontaneous circulation was achieved within 70 min of recirculation with CPB (Table 1). There were no differences between the groups in requirements of drug dosages during CPB, in the number of countershocks, or in the energy delivered to achieve restoration of spontaneous circulation. Three dogs in the trauma group could not be weaned from controlled

ventilation because of severe airway edema (resulting in upper airway obstruction) and spontaneous hypoventilation. Consequently, neurologic outcome was evaluated in these dogs at 72 h after reversing the neuromuscular blockade and analgesia with the orotracheal tube in place and, if necessary, with intermittent hand ventilation. They were then reanesthetized for perfusion fixation and morphologic evaluation.

Physiologic parameters

No significant differences were found in the baseline physiologic parameters between the two groups. Heart rate, MAP and cardiac output values were not different between the groups (Table 2). There were no group differences in arterial pH, PO_2, PCO_2 or base excess during the experiment (controlled parameters). An average flush volume of 620 mL/kg (range 360-800) was required to reach the target Tty of 10°C (Fig. 1). Lactate levels peaked in both groups 60 to 90 min after initiation of CPB without any group differences, and returned gradually back to baseline at approximately resuscitation time 6 h.

Coagulation and Blood loss

After initial exsanguination cardiac arrest there was minimal blood loss from skin incisions. To maintain hematocrit >25%, transfusion of donor blood was, however, required in 6 of 8 dogs in the trauma group and in no dog in the control group. Blood loss from the abdominal or thoracic injuries varied (Table 3). The transfusion volume varied between zero and 1500 mL and correlated with final neurologic deficit ($p = 0.039$). Both groups demonstrated coagulation abnormalities after the insult, with transient hypocoagulability by TEG (decreased alpha-angle and decreased maximum amplitude) at 1 h of recirculation (Table 4); TEG variables normalized

in both groups within 24 h, but indicated a hypercoagulable state at the end of the experiment (72 h), with an increased alpha-angle and large maximum amplitude.

Extracerebral outcome

At 72 h, in both groups, arterial pressure (Table 2) and blood gas values were normal and no dog required norepinephrine. At necropsy, moderate edema of subcutaneous tissue, airway mucosa and intestinal mucosa was observed in two trauma dogs and mild to moderate pleural effusions and ascites in 4 of the 8 trauma dogs (Table 5).

In the control group, no tissue edema and no other macroscopic extracerebral organ damage was observed at necropsy except for mild myocardial lesions (mainly focal subendocardial infarctions) in 3 of the 6 dogs. In 6 of 8 dogs in the trauma group, macroscopic cardiac damage was present, especially involving the anterolateral free wall of the right ventricle. In 2 of 8 dogs in this group, these lesions consisted of mild to moderate hemorrhagic infarctions mainly restricted to the subendocardium and subepicardium. In 4 trauma dogs, the heart surface lesions had coalesced and focally extended to transmural involvement. In the trauma group, total serum creatinine kinase (CK) significantly increased to 727 IU/L (range 447-1593 IU/L) but there was no significant increase in the CK-MB isoenzyme proportion and no increase in troponin-I levels, except for one dog with troponin-I of 8.8 ng/mL.

The lungs in both groups appeared normal, except hemorrhagic consolidation in one lower lobe in one trauma dog. The intestinal mucosa had mild to moderate hemorrhagic areas in 3 dogs in the trauma group. Anuria started in both groups with the onset of cardiac arrest and ended after 30-60 min of reperfusion, except in one trauma dog in which it persisted until 20 h; oliguria persisted in this dog as the creatinine level increased to 6.8 mg/dL and BUN to 66

mg/dL at 72 h. The kidneys had focal infarctions, edema or hemorrhage in 4 dogs in the trauma group. At 72 h, serum aspartate aminotransferase values were significantly increased in both groups (median 126, range 45-865 IU/L), whereas γ-glutamyl transpeptidase and bilirubin concentrations remained normal and serum albumin was below normal (median 2.3, range 2.1-2.5 g/dL).

Cerebral outcome

Final OPCs at 72 were better in the control group (Figure 2). All 6 control dogs were functionally normal (OPC 1). In the trauma group, 5 of 8 dogs were neurologically intact or had minor deficits (OPC 1 or 2). Three dogs in this group had poor neurologic outcome ($p = 0.208$): two remained comatose (OPC 4) and required controlled ventilation despite discontinuation of anesthesia, sedatives and analgesics and despite administration of naloxone and flumazenil; the third was re-intubated within an hour after extubation at 24 h due to stupor, general weakness and respiratory failure. The latter dog also remained on controlled ventilation until 72 h. Final NDS was normal in the control group (median 1, range 0-13) and abnormal in 4 of the 8 trauma dogs (median 12, range 0-87) ($p = 0.004$) (Fig. 2). Histologically, total brain HDS at 72 h was near normal in all dogs of both groups and averaged 12 (4-22) in the control group versus 0 (0-6) in the trauma group (NS) (Fig. 3). Regional brain HDS had the same distribution in both groups, with putamen and caudate nucleus being the most vulnerable regions. Histopathologic changes consisted mainly of scattered ischemic neurons in the vulnerable areas and, in 3 dogs, mild edema with no infarction.

DISCUSSION

In pilot experiments we found that exsanguination cardiac arrest of 90 min plus trauma is not reversible to intact survival, while without trauma full neurologic recovery could be achieved [12]. The cause of early post-arrest death in the trauma experiments with 90 min cardiac arrest, in spite of standard life support, was failure of multiple extracerebral organs, without significant brain damage. In the present definitive study, using a dog model of traumatic exsanguination cardiac arrest of 60 min, we found that rapid induction of profound hypothermia (suspended animation) can enable survival without brain damage, as we have shown without trauma before [12]. Although post-resuscitative extracerebral organ complications were worse in the trauma group, all the dogs survived to 72 h, 5 of 8 with good overall performance (OPC 1 or 2). Most importantly, no dog, with or without trauma, had any significant morphologic damage to the brain. This finding is important since, with conventional resuscitation techniques, the prognosis after traumatic exsanguination cardiac arrest is extremely poor [1,17,18]. The lack of histologic brain damage suggests that with longer intensive care life support (beyond 72 h), as is available clinically, all dogs might have achieved normal overall function in spite of trauma.

The concept of preserving the organism with suspended animation to buy time for transport and surgical repair with delayed resuscitation particularly applies to civilian or military trauma victims with truncal injuries who exsanguinate to cardiac arrest without concomitant brain injury. Such casualties are considered unresuscitable despite the fact that their injuries are technically repairable.

Systematic outcome studies in dogs have documented the feasibility of suspended animation for delayed resuscitation from cardiac arrest no-flow periods of up to 120 min [9-12]. The model used in these earlier studies with exsanguination through arterial and venous catheters

simulated isolated vascular injuries, without major tissue trauma. The majority of exsanguinating trauma victims, however, has concomitant injury to soft tissues and solid organs. Trauma may cause the systemic inflammatory response syndrome, including release of cytokines and soluble adhesion molecules, which is associated with the development of the multiple organ dysfunction syndrome (MODS) [19-21]. Moreover, coagulation disturbances associated with trauma, ischemia, hemodilution, hypothermia, cardiopulmonary bypass and reoxygenation injury may impact the outcome of operative intervention and may decrease the chance of achieving surgical hemostasis and long-term survival [22-25]. In the present model, all these pathophysiologic disturbances are associated with suspended animation, and may contribute to the development of severe coagulopathy and MODS. The extracerebral organ complications observed in the 3 dogs of the trauma group with poor outcome (OPC 3 and 4) are characteristic of MODS as defined by physiologic criteria [15,16]. Despite these dogs' poor overall and neurologic performance, however, no histologic damage was found in their brains. This discrepancy between histologic brain damage and clinical performance is in contrast with our results from previous cardiac arrest studies without trauma, in which a significant correlation has been seen between NDS and HDS [4,7-11,13,25-30]. Extracerebral organ dysfunction was, however, not present in the previous studies. Therefore, poor OPC and NDS scores in the trauma dogs of the present study may represent a metabolic encephalopathy, which is potentially reversible if the underlying derangement is corrected. These dogs continued to require ventilatory support. To tolerate this, they needed sedation and intermittent doses of a neuromuscular blockade. We cannot be certain that these effects were totally reversed before the 72 h evaluation of function.

The finding that the need for blood transfusion was associated with worse functional outcome suggests that common pathophysiologic mechanisms are involved in the initiation of

coagulation derangements and MODS. These derangements may include activation of coagulation cascades, an inflammatory response with the release of cytokines and an upregulation of adhesion molecule expression, as well as the oxidative stress caused by lipid or protein oxidation through intracellular free radical generation.

Limitations of this study include the small number of dogs used, which may not detect small differences in the analyzed parameters. Although our trauma model is clinically relevant, it does not represent the wide spectrum of tissue injury that may cause exsanguination cardiac arrest. In addition, intensive care was provided for a maximum of 72 hours while the three trauma dogs with poor outcome would have required intensive care beyond this period of time in the clinical practice. Accordingly, it is likely that prolonged intensive care would have led to good outcomes in these dogs.

Given evidence that suspended animation of one hour can be survived without evidence of histologic brain damage in spite of extracerebral trauma and MODS, clinical feasibility trials of suspended animation for victims of exsanguination cardiac arrest should be considered, starting in large trauma centers. Potential subjects would be trauma victims who have a mechanism of injury consistent with exsanguinating hemorrhage and lose a pulse just prior to, or after, arrival in the emergency department. These patients typically undergo a resuscitative thoracotomy. Rapid access to the descending aorta could be obtained directly and ice-cold saline could be flushed toward the heart and brain [9-12].

In conclusion, suspended animation in dogs, using aortic cold flush and delayed resuscitation with cardiopulmonary bypass, enables survival without brain damage after exsanguination cardiac arrest of 60 min no-flow, even in the presence of trauma. Extracerebral organ complications after resuscitation, however, are worsened by trauma.

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LEGENDS TO FIGURES

Figure 1. Tympanic membrane temperatures during exsanguination cardiac arrest of 60 min no-flow. Resuscitation was with cardiopulmonary bypass. Data are given as mean ± SD. No differences were observed between the groups.

Figure 2. Final overall performance categories (OPC 1-5, panel A) and neurologic deficit scores (NDS, panel B) at 72 h after exsanguination cardiac arrest with or without trauma. Each dot of OPC represents one dog. NDS is depicted in a box plot with the 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles.

Figure 3. Regional and total brain histologic damage scores (HDS) at 72 h after exsanguination cardiac arrest with or without trauma. Box plots display the 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles and outliers are plotted separately as circles (trauma group) or crosses (control group). Only scores above 0 are presented.

Table 1.
Resuscitation variables required for restoration of spontaneous circulation (ROSC)

Group	Trauma	Control
Countershocks, total number	1 (1-4)	1 (1-3)
Countershocks, total energy (J)	225 (150-700)	150 (150-450)
Time of ROSC*	43 (27-72)	47 (24-55)
Total Bicarbonate (mEq)	97 (56-210)	111 (50-258)
Total Epinephrine (mg)	0.9 (0.6-1.9)	1.5 (0.4-3.0)
Total Norepinephrine (mg)	1.9 (0-5.9)	2.7 (1.2-7.8)

Data are represented as median (range). *time after initiation of cardiopulmonary bypass. No differences were observed between the groups.

Table 2.
Physiologic variables in trauma and control groups during resuscitation from 60 min exsanguination cardiac arrest and suspended animation by aortic flush.

Time of Reperfusion	Mean Arterial Pressure (mmHg)		Heart Rate (beats/min)		Cardiac Output (L/min)	
	Trauma	Control	Trauma	Control	Trauma	Control
Baseline	110±16	116±16	118±13	121±13	3.1±1	2.6
5 min	77±20	65±19				
15 min	73±6	77±20				
30 min	83±26	93±16				
60 min	106±7	118±11	119±29	132±13		
90 min	123±14	120±18	124±28	136±30		
2h	115±19	118±16	143±16	139±5	3±1.1	3.7±1.1
3h	135±21	122±18	134±22	138±28	4.4±1.1	4.3±2.1
4h	133±13	135±16	134±16	128±23	3.8±1.2	3.9±1.3
6h	136±10	143±13	128±16	118±7	2.6±1	2.6±0.4
9h	128±16	127±16	124±26	115±25	1.7±0.9	2.4±0.4
12h	118±15	126±5	125±42	102±32	2.2±0.9	2.7±0.6
16h	107±11	123±12	138±43	113±37	3.1±0.4	2.7±0.3
20h	104±15	106±13	139±47	104±37	2.9±0.5	2.9±0.7
24h	98±11	93±11	138±41	90±28	3±0.7	

BL = baseline. Values are expressed as mean ± SD. No heart rate values available during cardiac arrest. No cardiac output values available during cardiopulmonary bypass. No differences were observed between groups.

Table 3.
Cumulative blood loss and transfusion volume (mL) in the trauma dogs.

Dog #	1		2		3		4		6		8	
Time of Reperfusion (h)	Blood loss	Transfusion	Blood loss	Transfusion	Blood loss	Transfusion	Blood loss	Transfusion	Blood loss	Transfusion	Blood loss	Transfusion
1											500	400
2									125		1150	1200
4									255	400	1250	
6	60		550		0		70			800		
9					350				570			
12			950	750			160		935			
18	115				750	550				1400		
24	176		1300		800	950			1275			
40	250					1400			1325	1500		
Major bleeding site			Chest		Abdomen				Chest		Abdomen	
OPC	1		3		4		2		4		1	

OPC=overall performance category. There was no significant bleeding in dogs 5 and 7, both of which had OPC 1 at 72h.

Table 4. Thromboelastography variables in trauma and control groups.

	Alpha-angle		Reaction time (min)		Coagulation time (min)		Maximum amplitude (mm)	
Time (h)	Trauma	Control	Trauma	Control	Trauma	Control	Trauma	Control
Baseline	60±13	60±9	6.3±2.0	6.3±1.2	10.7±7.8	8.9±2.1	54±12	60±9
1	*32±13	*42±19	*9.4±1.8	9.3±3.7	*20.0±11.1	*16.1±7.7	*37±11	*45±11
3	*49±16	*45±16	*8.1±2.2	9.9±6.0	*18.5±20.4	16.5±12.1	50±14	49±12
6	*42±15	*51±9	9.8±4.1	8.1±3.1	*21.8±18.6	*12.4±3.8	48±10	49±13
9	*42±18	*49±10	*9.3±4.2	8.4±4.4	13.1±3.9	12.7±6.4	*42±17	53±7
15	*45±9	*53±11	8.0±2.3	7.3±1.3	13.0±3.3	11.4±2.5	50±6	49±15
24	55±6	51±12	6.7±1	8.6±2.4	10.2±1.8	12.8±3.9	54±3	48±11
72	67±13	*71±3	5.9±3.2	7.0±1.4	8.0±4.3	8.8±1.8	68±7	70±3

*$p < 0.05$ versus baseline. No significant differences were observed between the groups. Values are expressed as mean ± SD.

Table 5. Gross extracerebral organ damage at necropsy (72 h) after 60 min exsanguination cardiac arrest and resuscitation.

Group	Dog number	Edema	Pleural effusion	Ascites	Heart	Lungs	GI	Kidney	Liver
Trauma	1	0	0	1	1	0	2	0	0
	2	0	0	0	2	0	1	0	0
	3	4	1	0	4	0	0	1	0
	4	0	0	0	0	0	0	0	0
	5	0	2	1	0	1	0	0	0
	6	0	0	0	4	0	0	3	0
	7	3	2	0	4	0	3	2	0
	8	0	3	0	0	0	0	2	0
Control	1	0	0	0	0	0	0	0	0
	2	0	0	0	1	0	2	0	0
	3	0	0	0	1	0	0	0	0
	4	0	0	0	0	0	1	0	0
	5	0	0	0	0	0	0	0	0
	6	0	0	0	2	0	0	0	0
Trauma group (n=8)		0 (0-4)	1 (0-3)	0 (0-1)	2 (0-4)	0 (0-1)	0 (0-3)	1 (0-3)	0
Control group (n=6)		0	0	0	0 (0-2)	0	0 (0-2)	0	0

1 = minimal, 2 = minor, 3 = moderate, and 4 = severe. Group values are expressed as median (range)

A-418

October 16, 2002
2:00:00 PM - 3:30:00 PM
Orange County Convention Center, Room 224 D

Survival without Brain Damage with Suspended Animation after Traumatic Exsanguination Cardiac Arrest of 60 Min in Dogs

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Resuscitation attempts after traumatic exsanguination cardiac arrest (Exs-CA no-flow) rarely succeed. In dogs with non-traumatic Exs-CA of 90 min we achieved intact survival by aortic cold flush at CA 2 min to tympanic temperature (Tty) <10°C. In the present study, we explore the hypothesis that additional trauma would worsen the chance of intact survival.

Using 16 pilot experiments we defined the trauma, hemorrhage and flush. The definitive study was with traumatic Exs-CA 60 min. Fourteen male dogs were randomized into a control group without trauma (n=6) and a trauma group (n=8) which received at start of CA standardized laparotomy, spleen transection, and thoracotomy; and during CA splenectomy. In both groups, starting at CA 2 min, flush of saline at 2°C into the femoral artery was initiated and continued until Tty of 10°C. Restoration of spontaneous circulation and assisted circulation were with cardiopulmonary bypass (CPB) to 2 h (with heparin bonded system), and mild hypothermia (Tty 34°C) to 12 h, controlled ventilation to 20 h, and intensive care to 72 h. Outcome was evaluated as overall performance categories (OPC 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, 5 = death); neurologic deficit scores (NDS 0-10% = normal, 100% = brain death); and 72 h perfusion fixation, necropsy, and determination of total and regional brain histologic damage scores (HDS). Hematocrit was kept >25, if needed with donor blood.

All 14 dogs survived to 72 h. The 6 non-trauma control experiments resulted in prompt resuscitation and intact survival (OPC 1), NDS 1% (range 0-13%) and total HDS 11 (4-22). In 3/8 trauma dogs controlled ventilation was needed beyond 20 h because of airway edema, hypoventilation, cardiovascular complications, renal failure and neurologic deficit. 4/8 trauma dogs achieved final OPC 1, one OPC 2, one OPC 3, and two OPC 4; NDS was 13% (0-87). Blood loss in the trauma group ranged widely (up to 1300 mls) and was associated with poor outcome.

Coagulation studies revealed in both groups, after resuscitation, transient initial hypocoagulation with coagulation factors consumption, and fibrinolysis activation. This was followed by delayed hypercoagulation. There was no evidence of sustained DIC. Platelet count decreased to 50% baseline at 1 h after resuscitation, without normalization by 24 h. Plasma concentrations of plasminogen activator inhibitor peaked at 6-9 h after the insult. All changes occurred in both groups, but were numerically worse in the trauma group.

We conclude that rapid induction of profound hypothermia (Tty 10°C) (Suspended Animation) can enable survival without brain damage after Exs-CA of 60 min no flow even in the presence of trauma, although with worse exracerebral organ failure. Coagulopathy and possibly a thrombotic microangiopathy, as a result of ischemia, CPB, hemodilution and hypothermia, appear worsened by trauma.

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October 16, 2002
2:00:00 PM - 3:30:00 PM
Orange County Convention Center, Room 224 D

Hypothermia Induced during Cardiopulmonary Resuscitation Increases Intact Survival after Prolonged Normovolemic Cardiac Arrest in Dogs

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Studies by us and others have documented improved cerebral outcome with mild hypothermia (34°C) induced after cardiac arrest (CA) and restoration of spontaneous circulation (ROSC). We hypothesized that in a simulated unresuscitable CA dog model, intact survival can be achieved if hypothermia is induced during prolonged cardiopulmonary resuscitation (CPR) steps A-B-C.
Twelve dogs (20-25 kg) were subjected to 3 min of CA no-flow with ventricular fibrillation (VF), followed by 7 min CPR Basic Life Support and 30 min of unsuccessful CPR Advanced Life Support (ALS) with DC countershocks, FiO_2 1.0 and epinephrine boluses. Dogs were randomly allocated into two treatment groups: a control group with normothermic VF (n=6, tympanic temperature [Tty] 37.5° C throughout) and a hypothermia group (n=6) which received at VF 20 min a venous flush of 20 ml/kg normal saline at 2°C followed by veno-venous extra-corporeal blood cooling (cetheters in superior and inferior vena cava) until cardiopulmonary bypass (CPB) was initiated at VF 40 min. ROSC and assisted circulation were with CPB to 4 h and then mild hypothermia (Tty 34°C) to 12 h, controlled ventilation to 48 h, and intensive care to 96 h. Outcome was evaluated as overall performance categories (OPC 1 = normal, 2 = moderate disability, 3 = severe disability, 4 = coma, 5 = death); neurologic deficit scores (NDS 0-10% = normal, 100% = brain death); and 96 h perfusion fixation, necropsy, and determination of total and regional brain histologic damage scores (HDS). Lowest Tty in the hypothermia group was 27°C (range 26-28°C). ROSC was achieved in all 12 dogs. In the control group, 1 dog survived to 96 h but remained comatose (OPC 4); and 5 dogs died during the intensive care period, the majority within 24 h, because of malignant arrhythmias and respiratory failure or vasopressor resistant shock; "best" NDS was 92% (range 92 - 98%). In the hypothermia group, 5 of the 6 dogs survived to 96 h with good neurologic outcome - OPC 1 (P=0.025) and NDS 0% (0-7%). HDS results are pending. In the control group there were renal failure and intestinal mucosal necrosis, severe subendocardial and epicardial hemorrhagic infarctions, and pulmonary infarctions. In the hypothermia group morphologic changes were absent or minimal (one with bilateral hemorrhagic pulmonary consolidations, 2 with mild subendocardial hemorrhage).
In conclusion, cooling during CPR attempts in prolonged normovolemic and presently unresuscitable cardiac arrest, as a bridge to prolonged CPB, results in survival with full neurologic recovery. A portable device for veno-venous cooling should be developed.
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PROTEOMIC CHANGES IN RAT BRAIN AFTER 30 MINUTES OF COMPLETE CEREBRAL ISCHEMIA WITH HYPOTHERMIA TREATMENT

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Introduction: Proteomic techniques offer unique potential to simultaneously evaluate changes in thousands of proteins in disease processes important to critical care, such as complete cerebral ischemia (CCI) secondary to cardiac arrest. Most studies of CCI have focused on reperfusion injury and resultant selective vulnerability. There has been only limited investigation of intra-ischemic neuronal responses. Hypothermia induced before or during prolonged cardiac arrest is an established neuroprotective modality. Our studies in dogs suggest 10°C to be maximally neuroprotective. **Hypothesis / Methods:** The purpose of the present study was to examine global hippocampal protein changes with 2D gel electrophoresis from rat brain subjected to CCI for 30 minutes without reperfusion at either 38°C or 10°C. The first dimension analysis was performed by isoelectric focusing with immobilized pH gradient (IPG) strips using tissue protein lysates from paired normo- and hypothermic rat hippocampi. Large (22x22 cm) SDS slab gels were run in the second dimension. Results: Approximately 500-600 protein spots/gel were found. About 5% of spots showed a 2-fold decrease in normothermia vs hypothermia after CCI. Spot matching with existing protein databases shows that changes in important cytoskeletal and cell signaling proteins were attenuated by hypothermia, suggesting specific targets for hypothermic neuroprotection in CCI. **Conclusions:** This preliminary study supports the feasibility of the use of proteomic techniques in the investigation of CCI and suggests that this powerful tool could provide important insight into the benefits of hypothermia. Parallel studies using Ciphergen protein chips are underway to identify potential protease cleavage products in these hippocampal samples. Supported by DOD grant # DAMD17-01-2-0038 and NS 35365.

Coagulopathy and multiple organ failure after traumatic exsanguination cardiac arrest (CA) of 60 min in dogs

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Supported by US Army contract DAMD17-01-2-0038

Introduction:

We could reverse CA of 120 min no flow at tympanic temperature (Tty) 10°C. Added tissue trauma caused coagulopathy and prevented survival.

Hypothesis / Methods:

Fourteen dogs were exsanguinated over 5 min to CA and then randomized into a control group without trauma (n=6) and a trauma group (n=8) which received at start of CA standardized tissue trauma. In both groups, starting at CA 2 min, centripetal flush of isotonic saline at 2°C into the distal aorta was to Tty 10°C; after 60 min CA, reperfusion was with cardiopulmonary bypass with heparin-bonded system. Intensive care was to 72h. Hematocrit was normalized in both groups with initially shed blood. In the trauma group fresh donor blood was added

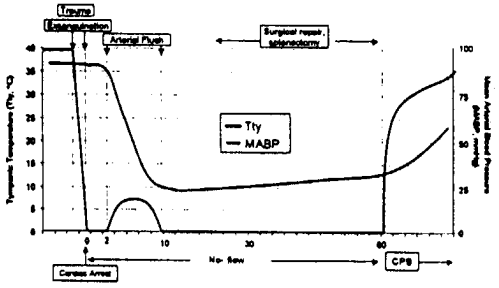


Fig. 1: Experimental protocol.

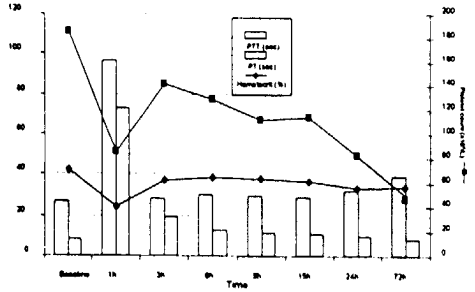


Fig. 2: Hematocrit, Platelet count, Prothrombin time (PT) and Partial Thromboplastin Time (PTT) at baseline and after reperfusion

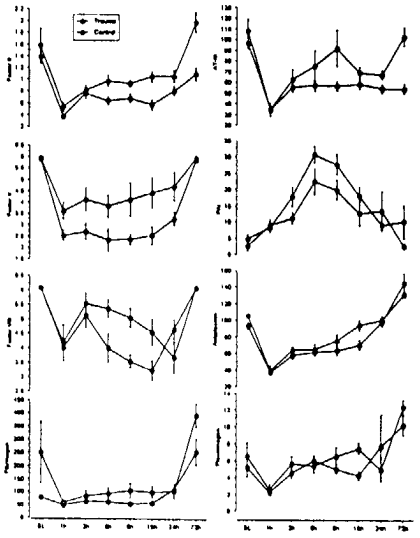


Fig. 3: Coagulation studies

	Trauma	Control
OPC 1 (normal)	●●●●	●●●●●●
OPC 2 (moderate disability)	●	
OPC 3 (severe disability)	●	
OPC 4 (coma)	●●	
OPC 5 (dead)		
NDS (%)	12 (0-87)	1 (0-13)
HDS	0 (0-6)	11 (4-22)

Fig. 4: Final 72-h outcomes. OPC= Overall Performance Category; NDS = Neurologic Deficit Score; HDS = Total Brain Histologic Deficit Score

Results:

All non-trauma dogs survived without neurologic deficits or extracerebral organ complications. In 3 trauma dogs, cardiovascular- pulmonary complications and renal failure occurred. Blood loss in the trauma group was 0-1300 ml and associated with poor outcome. At 1h of recirculation, TEG indicated in both groups severe hypocoagulation with narrowed alpha angle (α), prolonged reaction time (r) and reduced maximum amplitude (MA). PT and PTT were prolonged and factors II, V, VIII and fibrinogen levels reduced. AT-III levels were reduced and remained so until 24 h in the control group and until 72h in the trauma group. Platelet levels were 50% baseline at 1 h and did not normalize. PAI increased 6x at 6-9 h, with higher levels in the trauma group. It gradually decreased thereafter and was followed by a delayed hypercoagulation toward 72h, with wide ∝, short r and high MA in the TEG curves. At 72h, PT, PTT and clotting factors had normalized, but plasmin, antiplasmin and fibrinogen were increased.

Conclusion:

Cardiac arrest of 60 min no flow at 10°C is resuscitable, but represents challenges with complex coagulopathy, which is worse with than without trauma. The derangements suggest a thrombotic microangiopathy.

Intact survival in dogs after cardiac arrest (CA) of 40 min with mild hypothermia (34°C) during closed-chest CPR: myocardial and cerebral preservation

A Nozari, P Safar, X Wu, SW Stezoski, S Tisherman

Safar Center for Resuscitation Research, Depts. of Anesthesiology, Critical Care Medicine and Surgery, University of Pittsburgh, PA 15260

Supported by US Army contract DAMD17-01-2-0038

Introduction:

Mild hypothermia (34°C) *after* normothermic CA improves cerebral outcome. We hypothesized that mild or moderate hypothermia (30°C) *during* prolonged closed chest CPR steps A-B-C would further improve outcome.

Hypothesis / Methods:

Twenty-four dogs were subjected to ventricular fibrillation (VF), normothermic no flow of 3 min, basic life support of 7 min, and advanced life support (ALS) for unsuccessful defibrillation attempts of 10 min. They were then randomized to 4 groups: 1 (n=7) continued normothermic ALS; 2 (n=6) hypothermic flush (20 ml/kg normal saline IV at 2°C) and veno-venous extracorporeal shunt cooling to tympanic temperature (Tty) 26-28°C; 3 (n=6) same as group 2 but veno-venous shunt to Tty 34°C or 4 (n=5) normothermic flush and veno-venous shunt. After VF 40 min, reperfusion was with cardiopulmonary bypass. Intensive care was to 96 h. Outcome was evaluated as overall performance categories, neurologic deficit scores, and 96 h perfusion fixation, and determination of total and regional brain histologic damage scores (HDS). Groups 1 and 2 were presented before, group 3 and 4 are new.

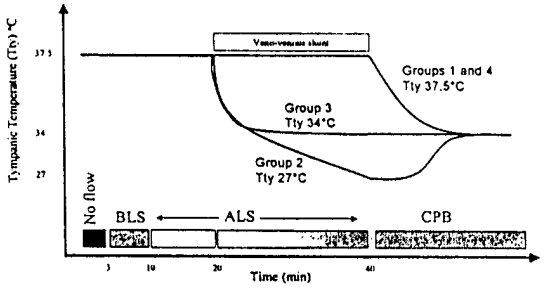


Fig. 1: Experimental protocol. BLS=Basic Life Support, ALS = Advanced Life Support, CPB = Cardiopulmonary By-pass

	Group 1 Control		Group 2 Tty 27°C		Group 3 Tty 34°C		Group 4 37.5°C	
	RV	LV	RV	LV	RV	LV	RV	LV
0 (absent)				●●●		●		
1 (minimal)			●	●●	●			
2 (mild)			●●●		●	●●●		
3 (moderate)	●	●●●			●●●	●	●	●●
4 (marked)	●●●	●●●	●●	●		●	●	●
5 (severe)	●●				●		●●●	●●
Pattern (1=focal, 4=diffuse)	4 (3-4)	4 (3-4)	2 (1-4)	0.5 (0-4)	3 (1-4)	2.5 (0-4)	4	4
Location (1=endocardial, 4=transmural)	3.5 (3-4)	3 (1-4)	1.5 (1-3)	0.5 (0-3)	2.5 (1-4)	1 (0-3)	3	3
Appearance 1=pale, 2=hemorrhagic	2	2	1.5 (1-2)	0.5 (0-2)	1.5 (1-2)	1 (0-2)	2	2

Fig. 2: Myocardial morphologic damage. RV = Right Ventricle, LV = Left Ventricle. Values are expressed as median (range).

	Group 1 Control	Group 2 Tty 27°C	Group 3 Tty 34°C	Group 4 Tty 37.5°C
Lungs	0 (0-5)	0 (0-3)	0 (0-1)	2 (0-3)
Kidneys	0 (0-3)	0.5 (0-3)	1.5 (0-4)	2 (2-3)
Liver	1 (0-4)	0 (0-3)	0.5 (0-3)	1 (0-3)
GI tract	0 (0-4)	0 (0-3)	1 (0-1)	4 (0-4)
Ascites	3 (0-4)	0 (0-1)	0	2 (2-4)

Fig. 3: Extracerebral Organ Damage. Values are expressed as median (range). 0=absent; 1=minimal; 2=mild; 3=moderate; 4=marked; 5=severe

	Group 1 Control	Group 2 Tty 27°C	Group 3 Tty 34°C	Group 4 Tty 37.5°C
OPC 1 (normal)		●●●●●	●●●	
OPC 2 (moderate disability)			●●●	
OPC 3 (severe disability)				
OPC 4 (coma)	●	●		
OPC 5 (dead)	●●●●●●			●●●●●
NDS (%)	92	1 (0-92)	1 (0-11)	
HDS	26 [78, 0]	1 (0-66)	0 (0-4)	[48]
MDS (%)	85 (73-97)	30 (13-87)	55 (27-80)	90 (80-93)

Fig. 4: Final 96-h outcomes. Values are expressed as median (range). OPC= Overall Performance Category, NDS = Neurologic Deficit Score, HDS = Brain Histologic Damage Score, MDS = Myocardial Macroscopic Damage Score (0 to 100% damage).

Results:

Of the normothermic CPR dogs, all in group 4 and all but one in group 1 (which remained comatose) died within 58 h, because of malignant arrhythmias and respiratory failure or vasopressor resistant shock. All dogs in the hypothermia groups (Tty 27 or 34°C) survived to 96 h, all but one with good neurologic outcome (OPC 1 or 2). Myocardial injury was present in all groups, but was less severe (degree) and less extensive (pattern) in the hypothermia groups (Fig. 2)

Conclusion:

Mild or moderate hypothermia *during* prolonged closed-chest CPR preserves viability of organs, without risk of complications, and improves outcome.

2003 Meeting of the National Neurotrauma Society
J Neurotrauma (in press)

THE EFFECTS OF HYPOTHERMIA ON RAT HIPPOCAMPAL PROTEOMIC PROFILES AFTER 30 MINUTES OF COMPLETE CEREBRAL ISCHEMIA

MS Chadha*[1,3], G Peters[2,3], X Zhang[1,3], P Safar[1,3], PM Kochanek[1,3], and LW Jenkins[2,3]. *1. Critical Care Medicine, 2. Neurological Surgery, 3. Safar Center for Resuscitation Research, U. Pittsburgh, Pittsburgh, PA, USA.*

2 dimensional (2D) gel electrophoresis is gaining momentum as a proteomic technique to evaluate neural injury. Because of inherent biological and technical variability in using this methodology to study brain selective vulnerability, there is a need to evaluate this approach with some rigor. Complete cerebral ischemia (CCI) without recirculation is an ideal model to evaluate homogeneous CNS changes since the ultrastructural responses vary little among different brain regions or cell types. Hypothermia is an established neuroprotective modality. This study was undertaken to assess the effect of hypothermia on rat hippocampal proteins during CCI and to test the sensitivity of a 2D gel based proteomic approach. After anesthesia and decapitation, hippocampi (n=6/group) were rapidly dissected and subjected to 30 minutes of decapitation ischemia at either 10 °C or 38 °C. To minimize genetic variability, only hippocampi from the same rat were compared. Isoelectric focusing with immobilized pH gradient (IPG) strips was coupled with large format (22x22 cm) slab gels for separation of proteins from hippocampal lysates. Spot matching with existing protein databases and selective MALDI-TOF mass spectrometry revealed significant hypothermic protection of key proteins fundamental to the regulation of energy metabolism and protein synthesis such as pyruvate dehydrogenase and eukaryotic initiation factor 2 beta, in addition to reduced cytoskeletal degradation. The use of a 2D gel based proteomic approach in the investigation of CNS injury, when combined with an appropriate injury model, can provide important insights. (Supported by NS 35365 and DOD grant # DAMD17-01-2-0038).

Society of Critical Care Medicine
33rd Critical Care Congress, February 20-25, 2004, Orlando, FL
In submission

Abstract # 62648

TITLE:
SUSPENDED ANIMATION AND PLASMA EXCHANGE (SAPEX) ENABLES FULL NEUROLOGIC RECOVERY FROM LETHAL TRAUMATIC EXSANGUINATION, EVEN AFTER 2H PERIOD OF NO-FLOW

AUTHORS (ALL): Nozari, Ala[1]; Safar, Peter[1]; Tisherman, Samuel [1]; Stezoski, William[1]; Kochanek, Patrick[1]; Wu, Xianren[1]; Kostelnik, Scott[1]; Carcillo, Joseph[1].

INSTITUTIONS (ALL): 1. Anesthesiology, Pediatrics, Surgery and Critical Care Medicine, Safar Center for Resuscitation Research, Pittsburgh, PA, USA.

ABSTRACT BODY:
Introduction: We have previously shown survival from lethal hemorrhagic / traumatic cardiac arrest (CA) after 1h of no-flow state using SA (4°C cooling within 2 min of CA). Survival beyond this period of no-flow is complicated with coagulopathy and multiple organ failure.

Hypothesis: PEX reverses coagulopathy and extends intact neurologic survival to 2h of no-flow.

Methods: Dogs were subjected to lethal hemorrhage (complete exsanguination), thoracic and splenic laceration and CA and a no-flow state. SA was performed with cooling to tympanic temperature of 10°C. Definitive surgery (laceration repair and splenectomy) was performed after 45 min of no-flow. After a total of 2 h of no-flow, SA was terminated and spontaneous circulation was restored using cardiopulmonary bypass at 34°C. PEX was performed in a randomized fashion thereafter (6, 20, and 40 h).

Results: Six dogs in the PEX group and 7 in the no-PEX group survived to 96h (p=NS). In the PEX group, 3 of 6 dogs were neurologically normal (overall performance category [OPC]1, p=0.03 versus no-PEX), 2 had weak hind legs (OPC2) and 1 could not walk (OPC3), and the median neurologic deficit score (NDS, 0=normal, 100=brain dead) was 6.5 (range 0-30). In the no-PEX group, 2 dogs were OPC2, 3 OPC3 and 2 OPC4 (severe disability) and the median NDS was 18 (range 9-53). Severe hypocoagulation occurred 2h after reperfusion (increased reaction time, decreased alpha angle and amplitude $p < 0.05$). Plasma exchnage therapy corrected the coagulopathy (PEX vs no PEX group, p=0.008).

Conclusions: Suspended Animation preserves previously lethal traumatic hemorrhagic victims for up to 2 hr when definitive surgical repair can be achieved. Subsequent use of PEX therapy reverses ensuant coagulopathy, enabling full neurologic recovery.

Character count 2010, limit 2200

Society of Critical Care Medicine
33rd Critical Care Congress, February 20-25, 2004, Orlando, FL
In submission

Abstract # 62622

TITLE:
SUSPENDED ANIMATION FOR 90 MIN CARDIAC ARREST IN DOGS WITH SMALL VOLUME ARTERIAL FLUSH AND VENO-ARTERIAL EXTRACORPOREAL COOLING

AUTHORS (ALL): Nozari, Ala[1]; Safar, Peter[1]; Stezoski, William[1]; Wu, Xianren[1]; Kochanek, Patrick[1]; Henchir, Jeremy[1]; Tisherman, Samuel[1].

INSTITUTIONS (ALL): 1. Anesthesiology, Pediatrics, Surgery and Critical Care Medicine, Safar Center for Resuscitation Research, Pittsburgh, PA, null.

ABSTRACT BODY:

Introduction: Suspended animation (SA) with rapid induction of profound hypothermia with a large volume flush into the aorta has resulted in intact survival after 90 min cardiac arrest (CA) no flow.

Hypothesis: A small flush volume to induce SA results in intact survival.

Methods: Twelve dogs (20-25 kg) were exsanguinated to CA over 5 min. At 2 min of CA, SA was induced by arterial flush using Plasma-Lyte at 2°C. In the Control-T group (n = 3), 400 mL/kg flush into the thoracic aorta was used to lower Tty to 15°C. In the Control-F group (n=3), Plasma-Lyte was infused into the femoral artery until Tty 15°C. In the Recirculation-T group (n = 3), 50 mL/kg aortic flush was followed by veno-arterial extracorporeal cooling until Tty 15°C. In the Recirculation-F group (n = 3), the flush was similar to the RT group but was into the femoral artery. Restoration of spontaneous circulation was achieved with cardiopulmonary bypass, and intensive care was given to 72 h.

Results: All dogs survived to 72 h, except for one in the CT group. No dogs in the one-way flush groups (CT and CF, n = 6) achieved normal overall performance category (OPC1), whereas 4 of 6 dogs were OPC1 in the recirculation groups (RT and RF, p = 0.061). Neurologic damage scores (NDS)varied according to OPC.

Conclusions: SA with a small flush volume and veno-arterial cooling to Tty 15°C enables intact survival with full neurologic recovery after 90 min of CA no flow. This method is clinically feasible and obviates the need for a large volume flush to induce SA, without worsening the outcome.

	CT group	CF group	RT group	RF group
OPC 5 (dead)	●			
OPC 4			●	
OPC 3	● ●	●		
OPC 2		● ●	●	
OPC 1 (normal)			●	● ● ●
NDS (0%=nomral, 100%=brain dead)	55,53	37,26,8	56,15,9	1,0,0

Character count 2193, limit 2200

1st Annual

Safar Symposium

Wednesday, November 20, 2002
Petersen Events Center

1st Annual Safar Symposium
November 20, 2002

Program

Morning Session

8:00 – 8:30	Continental Breakfast
8:30 - 8:35	Introduction of Chancellor Mark Nordenberg by John Williams, MD
8:35 – 8:45	Opening comments Mark A. Nordenberg, Chancellor
8:45 – 12:00	Novel Developments in Resuscitation and CNS Injury Research
Moderators:	Lyn Yaffe, MD and Patrick Kochanek, MD
8:45 – 9:05	Sam Tisherman, MD – Therapeutic Hypothermia in Resuscitation from Hemorrhagic Shock
9:15 – 9:35	Larry Katz, MD – Regulated Hypothermia and Neurointensive Therapy for Hypoxic Ischemia
9:45 – 10:05	Mark Angelos, MD – Postischemic Myocardial Dysfunction
10:15 – 10:30	Coffee break
10:30 – 10:50	Clif Callaway, MD – Effects of Hypothermia on Cellular Signaling After Brain Ischemia
11:00 – 11:20	Hülya Bayır, MD – Effective Hypothermia on Oxidative Stress After Severe Head Injury in Children
11:30 – 11:50	Larry Jenkins, PhD – Proteomics in Resuscitation Research
12:00 – 12:45	Lunch break

Afternoon Session

1:00	Introduction of Max H. Weil, MD, PhD by Mitchell Fink, MD
1:00 – 1:45	Speaker: Max Harry Weil, MD, PhD Cardiopulmonary Cerebral Resuscitation–Looking to the Future
2:00 – 4:30	The Role of Simulation in Resuscitation Research
Moderators:	Peter Winter, MD and Ake Grenvik, MD, PhD
2:00 – 2:20	Mr. Tore Laerdal Simulation: Needs and Opportunities in CPR Education
2:30 – 2:50	John Schaefer, MD Bringing Science to Medical Simulation Research
3:00 – 3:20	Daniel Swayze, EMT-P Myron Rickens, EMT-P Mobile Simulation
3:30 – 3:50	Michael DeVita, MD The Impact of Condition C Response and Crisis Team Training on Unexpected Death Rate in a University Hospital

Sponsored by:
The Departments of Anesthesiology,
Critical Care Medicine,
The Safar Center for Resuscitation Research
and the Winter Institute for Simulation, Education and Research

and

The U.S. Army Medical Research
and Materiel Command

SAFAR CENTER FOR RESUSCITATION RESEARCH

2001/2002 ANNUAL REPORT

DEPARTMENT OF CRITICAL CARE MEDICINE

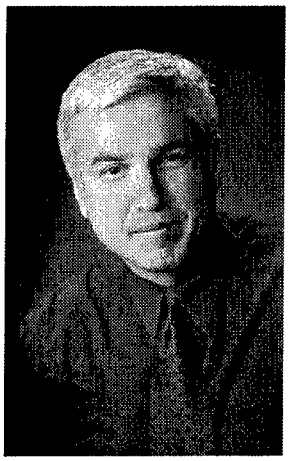
UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE

Table of Contents

Featured on the cover are photographs from the March 31, 2002 Sunday Magazine section of the Pittsburgh Post-Gazette. The article featured in that issue, entitled "The Beat Goes On," provided insight into the life's work of Peter and Eva Safar. *Top:* Dr. Peter Safar posing in front of an abstract painting of the girl who's death mask inspired the face of the Resusci-Anne manikin that is used worldwide to teach CPR. *Middle:* Dr. Safar amidst his files. *Bottom:* Peter and Eva Safar at the 2002 University of Pittsburgh Honor's Convocation.

MISSION STATEMENT

The global mission of the Safar Center for Resuscitation Research is to improve understanding of the mechanism of secondary injury after trauma and cardiopulmonary arrest, from whatever cause, and to contribute to the development and implementation of novel therapies. The treatment and prevention of secondary injury after these life-threatening catastrophic events is a major goal in each venue of investigation.



Patrick M. Kochanek, M.D. Director, Safar Center for Resuscitation Research

A letter from the Safar Center's Director

It is, once again, an honor to present the annual report of the Safar Center for Resuscitation Research. Our center has continued to flourish during the 2001-2002 academic year—its 22nd year of operation. The Safar Center is now formally a division of the new Department of Critical Care Medicine of the University of Pittsburgh School of Medicine. That department, chaired by Dr. Mitchell Fink is the first medical school based Department of Critical Care Medicine in the United States. That Critical Care Medicine has achieved departmental status in Pittsburgh is logical both on the basis of the unprecedented academic strength of the department and its historical status as the site of the nation's first multidisciplinary ICU and training program—pioneered by Dr. Safar in the late 1960s. I am pleased that the Safar Center is part of this new department.

The multidisciplinary nature of the Safar Center produces a unique and exciting environment for both trainees and faculty and the productivity and successes of the investigators and trainees continues to amaze me.

Three major areas of research and research training are in full swing and well funded—including research in traumatic brain injury, hemorrhagic shock and suspended animation, and training in neurointensive care, resuscitation, and rehabilitation research. Our traumatic brain injury program is funded by a program project grant from the National Institute of Neurological Disorders and Stroke, five RO-1s, one R-21 and KO-8, and a variety of other grants. It spans a number of areas of investigation—such as the study of novel resuscitative therapies targeting neuronal death, unraveling the mechanisms of secondary injury in both experimental models and in brain injured patients, development of novel tools to facilitate detection of occult cases of child abuse, and testing of new strategies in brain injury rehabilitation. We were all sad to see Dr. Donald Marion leave Pittsburgh. Don was a tremendous collaborator and integral member of the Safar Center family and he will be sorely missed. We wish him well in his new position as chairman of the Department of Neurosurgery at the Boston University School of Medicine. However, I am thrilled that Dr. C. Edward Dixon will assume the position of principal investigator of the program project. Ed is the perfect choice to continue to foster the outstanding collaboration that has developed between the Department of Neurological Surgery and the Safar Center. His strong links to the Department of Physical Medicine and Rehabilitation (PMR) will also

C. Edward Dixon, Ph.D. assumed the directorship of the University of Pittsburgh Brain Trauma Research Center.

help further unite these important components of the continuum of care in traumatic brain injury with the strong resuscitation and Critical Care medicine faculty within the Safar Center. The success of the link between PMR and our Center is further reflected by the fact that two PMR junior faculty members, Drs. Anthony Kline and Amy Wagner, now have sections within this report within the area of traumatic brain injury.

The hemorrhagic shock and suspended animation program—guided by Drs. Peter Safar and Samuel Tisherman also continues to break important new ground in the area of trauma resuscitation. This program continues to be well supported through congressional plus-up funding via the United States Army and a grant from the United States Navy. Experiments in this program have routinely achieved intact survival after exsanguination cardiac arrests of 90 minutes in duration, and in some cases, after two hours. This work is breaking new frontiers in cerebral preservation and resuscitation research. It is an honor to be able to watch the genius of Peter Safar as he carefully crafts this important project into the masterpiece that it has become. Consultative and administrative support from Dr. Lyn Yaffe, former director of the United States Naval Medical Research Institute has been instrumental to the success of this program, as has been the enthusiastic support of Col. Dean Calcagni and Robert Read of the United States Army.

Research training continues to be a key priority in our center –both postdoctoral fellow (MD and/or PhD) and junior faculty development. This represents the most important part of my own efforts. Postdoctoral clinician-scientist developments in the field of pediatric critical care medicine is greatly facilitated by our T-32 grant from the National Institute of Child Health and Development entitled "Training in Pediatric Neurointensive Care and Resuscitation Research." I wish to thank Drs. Ralph Nitkin, Michael Weinrich, Carol Nicholson, and Beth Ansel at NICHD for their valuable insight and support of this important program. We have also received funding from the Charles Schertz Grant from our Department of Anesthesiology. I cannot say how pleased I am to work with Dr. John Williams, Chairman of the Department of Anesthesiology, to ensure that the multi-departmental mission of the Safar Center continues to flourish. Finally, some postdoctoral fellowship positions are supported by individual faculty grants. Junior faculty development is supported by a number of grants, including KO8 awards to Drs. Robert Hickey in the division of pediatric emergency medicine (mentored by Dr. Steven Graham in the Department of Neurology) and Amy Wagner (mentored by Dr. C. Edward Dixon) in the Department of PMR. Also, Dr. Rachel Berger, in the Department of Pediatrics has submitted a K-23 award entitled "Using Biochemical Markers to Detect Abusive Head Trauma" (mentored by Dr. Kochanek) and we are

Rachel Berger, M.D. is carrying out an important study at Children's Hospital of Pittsburgh using serum biomarkers in an attempt to identify infants who are unrecognized victims of inflicted childhood neurotrauma (shaken baby syndrome).

optimistic that Rachel's important work will also be funded. Productivity by the trainees continues to be spectacular, including a total of 25 fellow first-author peer-reviewed publications this academic year. Several fellows received awards. Dr. Hülya Bayır received the Scientific Award from the Society of Critical Care Medicine during the 2001 Critical Care Congress for her work showing a marked gender effect of lipid peroxidation after traumatic brain injury in adult patients. Also, Dr. Berger received the Ambulatory Pediatric Association Fellows Award from the Ambulatory Pediatric Association for her work on the astrocyte marker S100B in pediatric head injury. The successful development of academic faculty in intensive care, resuscitation, and rehabilitation-relevant fields is our most important mission. I am proud of our successes on this front. Seven of our recent trainees have successfully competed for K awards from NIH and three of these individuals have gone on to achieve support as principal investigators at the RO-1 level.

Investigators in the Center published 59 peer-reviewed papers, 23 chapters, 48 abstracts, and 5 editorials during the 2001-2002 academic year. Included among these reports were publications in the *Journal of Cerebral Blood Flow and Metabolism, Pediatrics, Critical Care Medicine*, the *Journal of Neurotrauma, Shock, Brain Research, Pediatric Research, Neuroscience Letters, NeuroReport, Contemporary Neurosurgery, Journal of Trauma, Neurosurgery Clinics of North America, New England Journal of Medicine, Neuroscience, Journal of Neurochemistry, Resuscitation, Pediatric Neurosurgery, Academic Emergency Medicine, Pediatric Critical Care Medicine, Current Opinion in Anesthesiology,* and *Critical Care Medicine*. Particularly noteworthy publications included invited reviews authored by two of our T-32 fellows--Dr. Kimberley Statler in the *Journal of Neurotrauma* entitled "The Simple Model versus the Super Model," and Dr. Trung Nguyen in *Pediatric Critical Care Medicine* entitled "Microvascular Thrombosis in Pediatric Multiple Organ Failure—Is it a Therapeutic Target?" Dr Kochanek and co-authors published an invited review on "Cerebral Resuscitation after Traumatic Brain Injury and Cardiopulmonary Arrest in Infants and Children in the New Millennium" in the journal *Pediatric Clinics of North America.* Two medical students published a first author manuscript in *Pediatric Critical Care Medicine*--Jonathan Amick and Kristen Yandora, and a high school summer student, Sumeeta Varma, now at Stanford University--gave an impressive presentation of her paper entitled "Lipid Peroxidation after Severe Traumatic Brain Injury in Infants and Children: Assessment of F_2-isoprostane" at the annual meeting of the Society of Critical Care Medicine. Fellow, Dr. Wilhelm Behringer, under the mentorship of Drs. Safar and Tisherman, authored an important report on the suspended animation project–describing survival of 30 minutes of cardiac arrest with cooling by aortic flush in dogs—in the journal *Anesthesiology*. Dr. Safar's paper of 1958 in *JAMA* was selected as the first "classic paper" in a new series in *Anesthesiology*.

Michal Schwartz, Ph.D. was the 2000 Peter and Eva Safar lecturer and discussed her pioneering work in the area of protective autoimmunity after CNS injury.

The 2001 Peter and Eva Safar Lecturer was Michal Schwartz, Ph.D., who gave a provocative talk on "Protective

Autoimmunity after CNS Trauma and in Chronic Neurodegenerative Disorders: A Paradigm Shift." Professor Schwartz was born in Tel Aviv, Israel. She received a B.Sc. in chemistry from The Hebrew University of Jerusalem in 1971 and a Ph.D. in chemical immunology from the Weizmann Institute of Science, Rehovot, Israel, in 1977. In 2000, Professor Schwartz was named Career Woman of the Year in Israel. She is the first woman to be invited to deliver the Peter and Eva Safar Lecture at the University of Pittsburgh School of Medicine.

Visiting Professor to the Division of Pediatric Critical Care Medicine was Jacques R. Lacroix, M.D., Associate Professor of Pediatrics and Director of the Pediatric Critical Care Medicine Program at the University of Montreal, Sainte-Justine Hospital gave a lecture on "Red Blood Cell Transfusion: The Good, the Bad and the Ugly." Similarly, our critical care medicine and Safar Center fellows presented their research to him on the second day of his visit, and his suggestions to them were outstanding.

From left to right Safar Center research fellows, Ala Nozari, M.D., Kimberly Statler, M.D., Margaret Satchell, M.D., Pak Chan, Ph.D. (annual Safar Center visiting professor), Xianren Wu, M.D., Hülya Bayır, M.D., Trung Nguyen, M.D., and Margaret Wilson, Ph.D, after their scientific presentations to Dr. Chan during his visit. Dr. Safar's watchful eye is appropriately in the background.

Our annual visiting professor to the Safar Center was Dr. Pak Chan from Stanford University School of Medicine, Dept. of Neurology and Neurological Sciences. Dr. Chan lectured on "Oxidative Signaling as Molecular Switch for Cell Death or Survival in CNS Injury." Each of our fellows also presented their work to him for critique on the second day of his visit. His presentation and comments to our young investigators was extremely helpful and greatly appreciated.

Beginning on July 1, 2002, Dr. Clifton Callaway, a faculty member in the University of Pittsburgh Center for Emergency Medicine will take over from Dr. Nicholas Bircher as the director of the cardiopulmonary arrest program at the Safar Center. Clif is a talented young investigator, and I am optimistic that we can work together to cultivate an important interaction between the Emergency Medicine Department (chaired by Dr. Paul Paris) and the Safar Center. I would like to thank Nick for his longstanding dedication to cardiopulmonary resuscitation research at the Safar Center. I have revamped some of the programs and named several new directors—to reflect the current composition of our Center. Dr. C. Edward Dixon has taken over as the director of the new Functional Outcome Core, while Drs. Robert Clark and Larry Jenkins have taken over as co-directors

of the Molecular Biology Core of the center. These titles are long overdue for Ed, Bob, and Larry, who are both talented scientists and irreplaceable colleagues and friends.

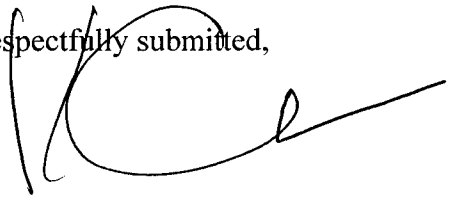
Once again, I would like to thank everyone working at the Safar Center for a terrific job this year. I am personally indebted to Linda Amick and Marci Provins for their administrative and secretarial excellence, respectively. Linda and Marci are extremely dedicated to the Safar Center and its success. Linda continues to take on an increasingly greater administrative role on the business end of the center while Marci serves as our key secretarial resource for the academic programs in our center –along with her incredible work as my local editorial assistant for the journal *Pediatric Critical Care Medicine*. I would also like to personally thank Henry Alexander, John Melick, Keri Janesko, Xiecheng Ma, Fran Mistrick, Ray Griffith, Jackie Pantazes, Grant Peters, and S. William Stezoski, who were senior administrative and technical staff members during the 2001-2002 academic year for their spectacular contributions to the individual missions of the Center. I continue to be amazed by the dedication and work ethic of these individuals and all of the technical and secretarial staff at our Center.

I would like to thank Dr. Mitchell Fink for his support as the new Chairman of the Department of Critical Care Medicine and I look forward to working with Susan Stokes, the new departmental administrator. I would like to thank Drs. Robert Clark, C. Edward Dixon, Larry Jenkins, Donald Marion, Ross Zafonte, Clifton Callaway, Nicholas Bircher, P. David Adelson, Xiaopeng Zhang, Anthony Kline, Amy Wagner, Hong Qu Yan, and of course Peter Safar for their camaraderie and guidance with the continued development of the Safar Center and its programs. I would also like to thank Dr. John Williams, Chairman of the Department of Anesthesiology, for his interest in our Center.

Special thanks are also due to Dr. Chien Ho and Kristy Hendrich at the Pittsburgh NMR Center for Biomedical Research, Dr. Edwin Jackson in the Center for Clinical Pharmacology, Dr. Valerian Kagan in the Department of Environmental and Occupational Health, Dr. Stephen Wisniewski in the Department of Epidemiology, Dr. Rachel Berger in the Department of Pediatrics, Dr. Timothy Carlos in the Department of Medicine, Dr. Simon Watkins in the Department of Cell Biology and Physiology, Dr. Timothy Billiar in the Department of Surgery, Dr. Paul Paris in the Department of Emergency Medicine, Dr. David Perlmutter in the Department of Pediatrics, and Dr. Melvyn Heyes at the Curagen Corporation for outstanding collaborative expertise that raises the level of the research at the Safar Center.

I once again look forward to success in 2002-2003 in our investigative efforts to develop new therapies in the field of resuscitation medicine.

Respectfully submitted,



Patrick M. Kochanek, M.D.

Patrick M. Kochanek, M.D., Director, Safar Center for Resuscitation Research
Director, Traumatic Brain Injury

Peter J. Safar, M.D., Distinguished Professor
Director, Shock and Suspended Animation

Samuel A. Tisherman, M.D.
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Anthony Kline, Ph.D.
Donald Marion, M.D.
Ernesto A. Pretto, M.D.
S. William Stezoski
Amy Wagner, M.D.
Xiaopeng Zhang, M.D.

Guest Scientists
Rachel Berger, M.D.
Steven DeKosky, M.D.
Howard Ferimer, M.D.
Robert Garman, D.V.M.
Steven Graham, M.D., Ph.D.
Kristy Hendrich, B.S.
Robert Hickey, M.D.
Sam Poloyac, Ph.D.
James V. Snyder, M.D.
Hong Qu Yan, M.D.

Visiting Scientists
Ann Radovsky, D.V.M.
Lyn Yaffe, M.D.

Fellows
Hülya Bayır, M.D.
Mandeep Chadha, M.D.
Yong Y. Han, M.D.
Trung Nguyen, M.D.
Ala Nozari, M.D.
Margaret Satchell, M.D.
Paul Shore, M.D.
Kimberly Statler, M.D.
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Vince Vagni

Students
Aaron Gordon
Laura Griffith
Danielle Kausler
Jaime Massucci
Nicole Williams

Funding

During the 2001-2002 academic year, Safar Center investigators had a total of 48 active grants. 43of these grants were extramural. The direct and indirect costs for the full award period of these grants totaled $18,908,318 and this is plotted for the current and preceding four academic years on the following page. The specific sources of this grant support are shown on the subsequent page. Remarkably, the Safar Center is continuing to grow and maintain a high level of extramural support.

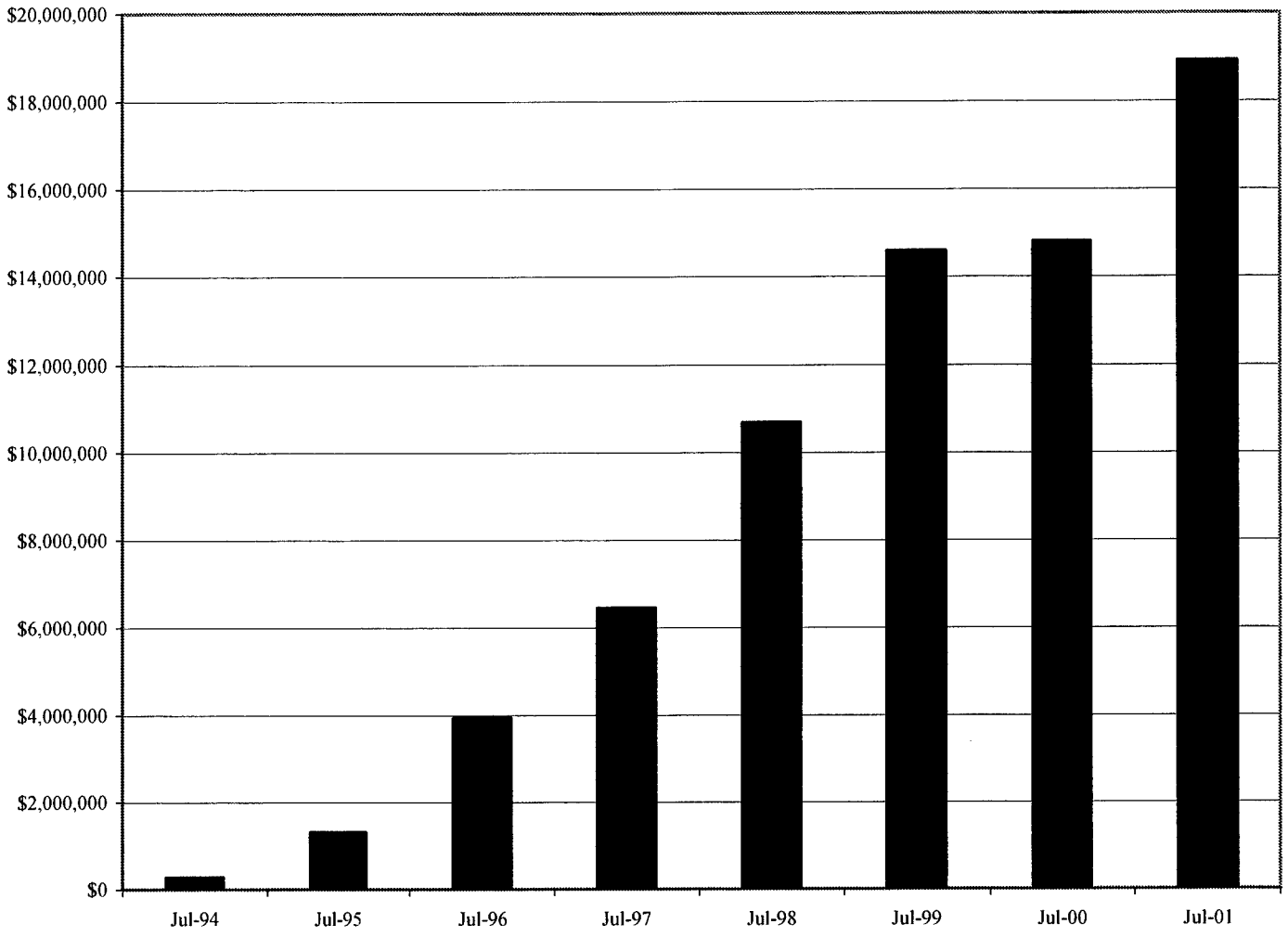
The portion of the budget for use in each academic year (July 1 through June 30) is also plotted for the current and preceding four academic years on the pages following. This represents direct and indirect costs and is shown for total, extramural, and intramural grant support.

Extramural funding sources included the National Institutes of Health, the United States Army, the United States Navy, the Centers for Disease Control and Prevention, the Laerdal Foundation, and a variety of other sources including The Pittsburgh Foundation. Contributions were made to the Safar Center in memory of Eric Bundy.

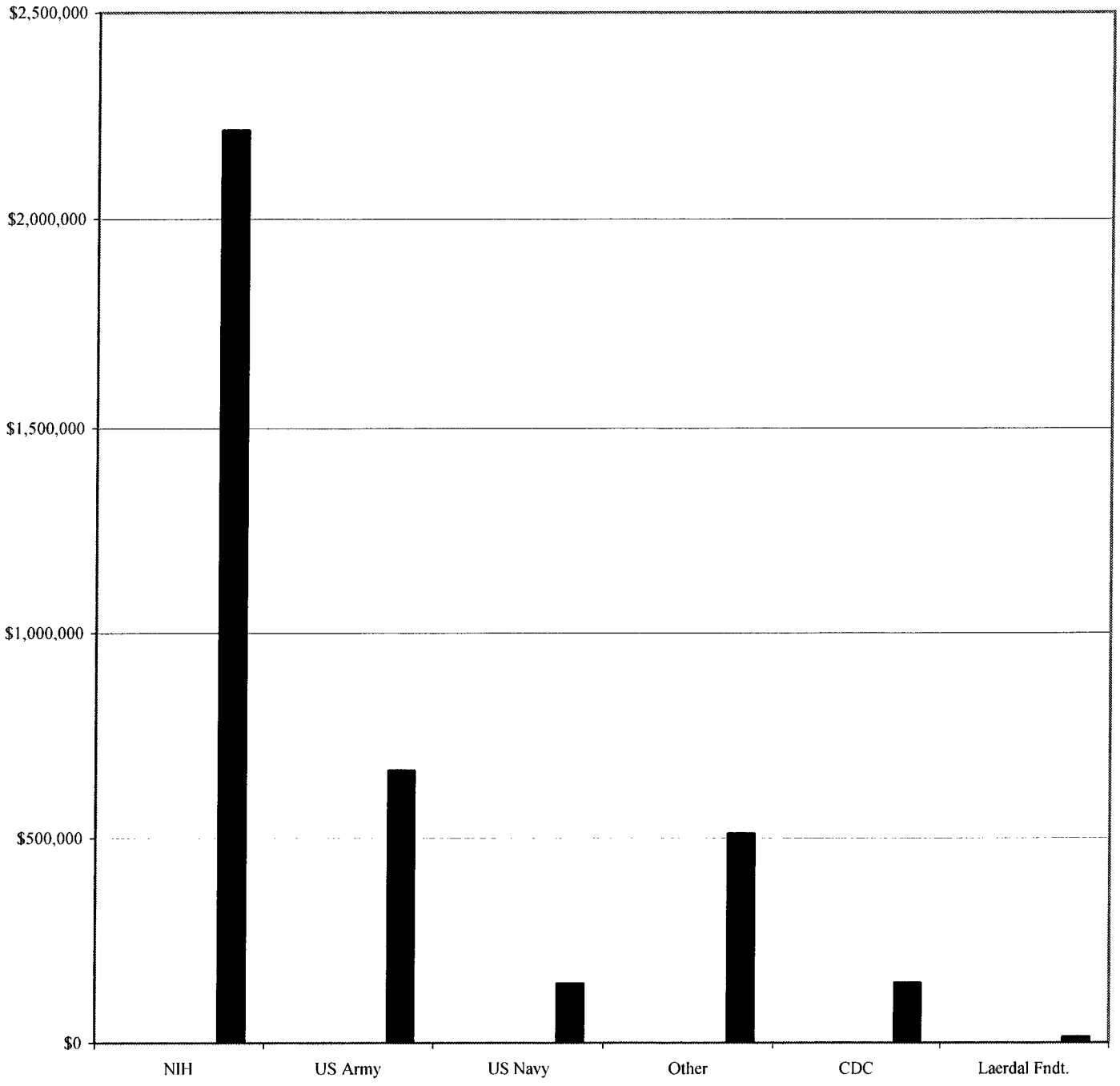
Intramural funding was provided by the Departments of Anesthesiology, Critical Care Medicine, Children's Hospital of Pittsburgh, and the Pittsburgh Mercy Foundation, Mercy Hospital of Pittsburgh.

We are deeply grateful for the prior and current support from all of these granting agencies and donors.

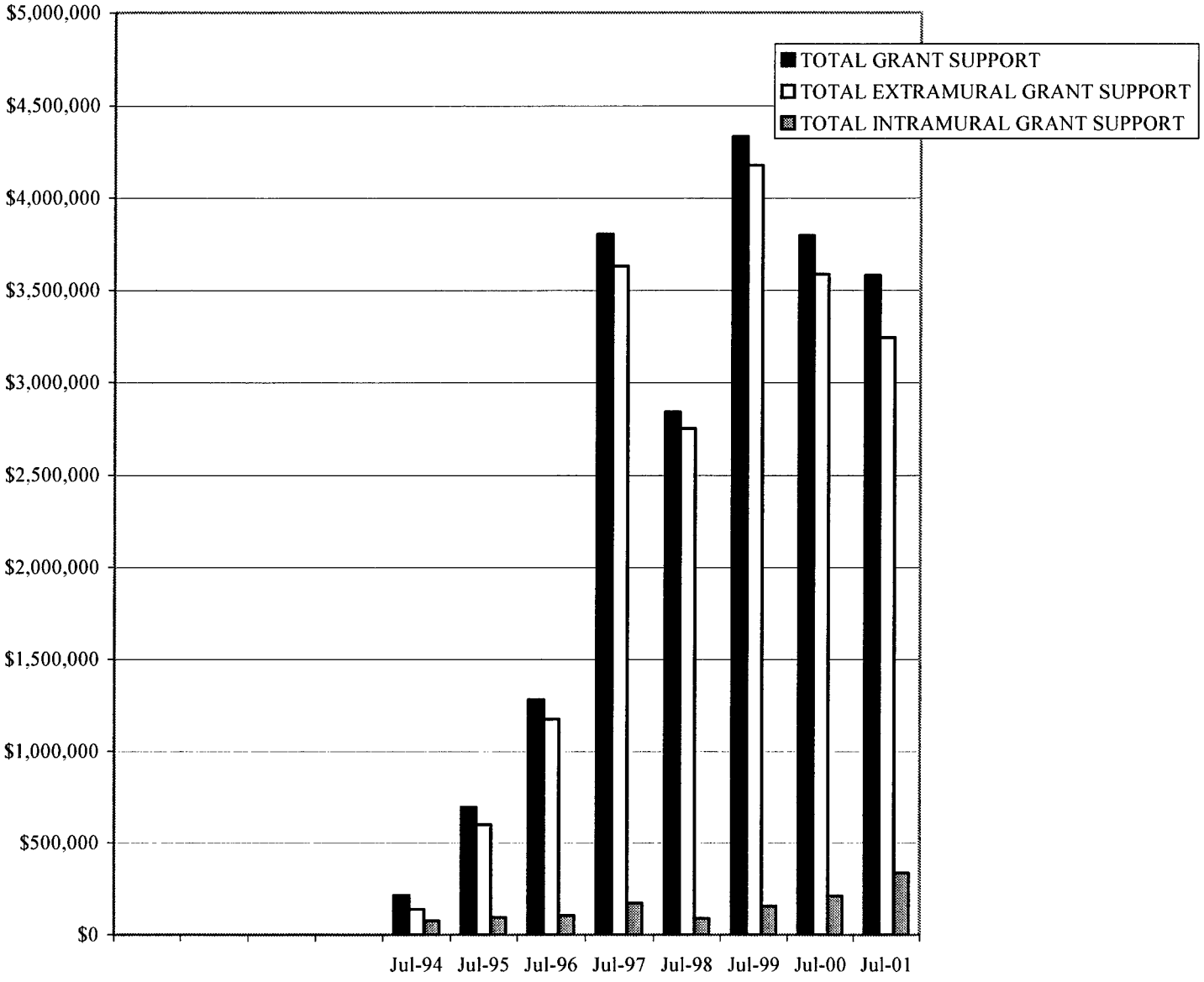
Direct and Indirect Costs for the Full Award Period of SCRR Grants



Specific Sources of Grant Support



Safar Center Grant Support thru 2001/2002
use in each academic year



TRAUMATIC BRAIN INJURY (TBI) PROGRAM

Research in TBI at the Safar Center is accomplished through a collaborative effort between a number of investigators, fellows, students and staff located principally in the Department of Critical Care Medicine (CCM), including the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), Neurosurgery, and Neurology at the University of Pittsburgh School of Medicine. A large number of collaborations are also ongoing with investigators in other University of Pittsburgh Departments including the Center for Clinical Pharmacology, Environmental and Occupational Health Medicine, Neurological Surgery, Pediatrics, Neurology, Epidemiology, Anesthesiology, and Surgery. In addition, a long-standing collaboration is in place with the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University. We have also had a number of important extramural collaborators, Dr. Melvyn Heyes at the Curagen Corporation, Dr. Naoto Minamino at the National Cardiovascular Center Research Institute in Osaka, Japan, Dr Jiang-Fan Chen at the Harvard Medical School, Dr. Jurgen Schnermann at the NIH, and Dr. Ann-Christine Duhaime at Dartmouth University. Taken together, these collaborations have allowed us to investigate a broad spectrum of mechanisms that may be important to the evolution of secondary damage after TBI. Our most important work continues to be in the area of defining the mechanisms important to secondary brain injury both after experimental TBI and in the human condition. Our studies of mechanism of secondary damage and repair in human materials (cerebrospinal fluid [CSF], brain tissue samples from resected contusions, and microdialysis samples) have generated new insight into the biochemistry and molecular biology of human head injury. Based on this mechanistic work, we are currently testing novel therapies in our experimental models. Our goal is to develop new therapies that can be successfully translated to clinical application. Our clinical research of taking the bench to the bedside—particularly as it relates to child abuse—has been featured many times in the lay press.

1. Studies directed by Patrick M. Kochanek, M.D.

A. Biochemical Assessment of Secondary Mechanisms of Injury and/or Repair after Severe TBI in Infants and Children: The Role of Child Abuse.

This continues to be an important area of research for our group and, as indicated above, continues to generate considerable publicity. We are using samples of CSF and blood collected from infants and children suffering severe TBI to study a variety of biochemical mediators of secondary damage and/or repair. These samples are collected by Dr. Rachel Berger in the Department of Pediatrics and member of our critical care team including Drs. Robert Clark, Hülya Bayır, Paul Shore, Randall Ruppel, Yi-Chen Lai, Mandeep Chadha, and Erica Fink in the division of Critical Care Medicine, Dr. Rachael Berger, in the Department of Pediatrics, and Dr. David Adelson in the division of Neurosurgery at Children's Hospital of Pittsburgh. To generate a CSF bank for this purpose, Dr. Kochanek is funded by the CDC (University of Pittsburgh Center for Injury Control and Research/CIRCL). We have now over 1000 samples from nearly 100 infants and children

who have suffered a severe TBI—including over 20 victims of inflicted TBI (shaken baby syndrome). In addition, we continue to collaborate with Dr. Neal Thomas at the Hershey Medical Center, Hershey, PA, who is also collecting samples.

Studies using the pediatric CSF bank at the Safar Center

The pediatric CSF bank and related clinical projects have produced some of the most interesting findings in the area of TBI at the Safar Center in the 2001-2002 academic year. Work has progressed in seven major areas including 1) oxidative stress, 2) detection of occult inflicted childhood neurotrauma, 3) adenosine and related metabolites in TBI, 4) markers of neuronal death study, 5) growth factors and markers of regeneration and repair, 6) studies of the effect of hypothermia on markers of secondary damage after TBI, and 7) assessment of the effect of the mode of CSF drainage in pediatric TBI.

Oxidative stress in TBI

This is an exciting area of research spearheaded by Dr. Hülya Bayır, a senior PICU fellow and the 2002 Charles Schertz Fellow in the Department of Anesthesiology. Dr. Bayır's work entitled "Assessment of antioxidant reserve and oxidative stress in CSF after severe TBI in infants and children" was published as a full paper in *Pediatric Research.* That work, done in collaboration with Dr. Valerian Kagan, provided substantial evidence for oxidative stress in brain after severe TBI in infants and children. Dr. Bayir followed up on that study an equally important report entitled "Effect of hypothermia on oxidative stress after TBI in humans: a preliminary report" that she presented at the 2001 meeting of the National Neurotrauma Society and will present at the 2002 meeting of the SCCM. She has begun to use the battery of markers of oxidative stress and damage that she has developed with Dr. Kagan, to evaluate the effect of therapies—including moderate hypothermia in adults. Current studies by Dr. Bayır of the effect of hypothermia on oxidative stress in pediatric TBI are also underway—done in conjunction with the RCT being carried out by Dr. Adelson at Children's Hospital of Pittsburgh. Dr. Bayır has also worked under the direction of Dr. Kagan to study a novel marker of nitrosative stress in pediatric TBI, namely, S-nitrosylation. Their work in this novel area was presented at the 2001 meeting of the Society for Neuroscience and a full manuscript is *in press* in the *Journal of Cerebral Blood Flow and Metabolism.*

Detection of occult inflicted childhood Neurotrauma

This important area of research originated from a small grant awarded to Dr. Kochanek within the University of Pittsburgh Center for Injury Control and Research (CIRCL) focused on the use of inflammatory markers in CSF as a biological clock to provide insight into the timing of injury in infants who were victims of the shaken baby syndrome. Often these infants are either chronically injured, or there may be a delay in presentation. In the past year, Dr. Rachel Berger, a general pediatrician working in the area of child abuse at Children's Hospital of Pittsburgh, has done an exemplary job in broadening the potential relevance of this project by studying the potential use of serum markers of brain injury with the hope of detecting otherwise unidentified brain injury in possible victims of child abuse. Rachel first showed that CSF levels of markers of neuronal (Neuron specific enolase [NSE]), glial (S-100B), and axonal (myelin basic

protein) were massively increased versus control after severe TBI in infants and children—including child abuse victims. That work was published this year in the journal *Pediatrics*. She also published an important report in the *Journal of Neurotrauma* showing that these markers of brain injury are increased in serum in over one-third of infants and children with mild TBI—children that are often sent home from the emergency department. This study has set the stage for an assessment of the use of these biomarkers in a target population of infants in diagnostic categories that occasionally represent missed cases of abusive head injury—such as vomiting without diarrhea, a seizure without fever, unexplained bruising, etc. That study is the centerpiece of Dr. Berger's recently submitted K-23 award—that we are optimistic will be funded by NICHD this year. Rachel is quickly becoming a leading investigator in this area. Relevant to this area, Dr. David Adelson was the co-editor of an issue of *Neurosurgical Clinics of North America* that was devoted to child abuse and several Safar Center faculty and fellows were authors on review papers in that issue.

Adenosine in TBI

Dr. Kochanek is beginning year-4 on an RO-1 from NINDS focused on adenosine in TBI. Translational work is an important part of this effort and the CSF bank represents a key resource. Dr. Courtney Robertson's article on CSF adenosine in pediatric TBI (see last year's report) was published this year in the journal *Critical Care Medicine*. Investigation of the effect of hypothermia on adenosine and purine related markers of energy failure are ongoing in collaboration with Dr. Edwin Jackson. Similarly, Ava Puccio is evaluating the relationship between CSF adenosine and tissue oxygen levels in adults with severe TBI, in work done with Dr. Marion at Presbyterian Hospital.

CSF markers of neuronal death in TBI

As part of the impressive work of Dr. Robert Clark's group on mechanism of neuronal death, including studies on caspases, apoptosis inducing factor (AIF), and poly ADP ribose polymerase (PARP), translational studies are similarly taking advantage of our CSF bank and brain tissue samples. Drs. Margaret Satchell and Xiaopeng Zhang have published a series of abstracts on PARP activation and protein kinase B signaling after TBI in humans. Some of Dr. Satchell's work was discussed in last year's annual report. This translational approach is providing important human data on contemporary and intensively investigated mechanisms of neuronal death in experimental TBI—and has the potential to help guide the development of novel therapies.

Growth factors and markers of regeneration and repair

Building on the prior work of both Dr. Steven DeKosky on nerve growth factor, and Edwin Jackson on the relationship between adenosine A2β receptor activation and elaboration of vascular endothelial growth factor (VEGF), Dr. Paul Shore presented a paper at the 31st Congress of the SCCM reporting marked increases in VEGF after severe TBI in infants and children. At the same meeting, Dr. Erica Fink, a senior pediatric resident working with Dr. Clark reported increases in hepatocyte growth factor in CSF after injury. We have been struck by the robust and rapid regenerative response that occurs after TBI and by the fact that this is readily detected using CSF.

Effect of modes of CSF drainage
Dr. Paul Shore is carrying out a comparative study (in collaboration with Dr. Neal Thomas at Hershey Medical Center) assessing the effect of continuous versus intermittent CSF draining on mediator levels and pathophysiology after severe TBI in infants and children. We are pleased to collaborate with Dr. Thomas, a former fellow in our program, in this study that addresses a basic treatment approach (CSF drainage) that has been subjected to remarkably little investigation.

Our pediatric CSF bank continues to represent a key research tool of our trainees to help bring the bench to bedside in the study of secondary injury mechanism in clinical TBI research.

Support: Quinolinic Acid in Cerebrospinal Fluid Early after Severe Head Injury in Victims of Child Abuse R49/CCR310285-03, (9/1/01-8/31/02), $45,110, Patrick Kochanek, PI, Melvyn Heyes, Ph.D., [Curagen Corporation], Stephen Wisniewski, Ph.D., Donald Marion, M.D., and P. David Adelson, M.D., Co-investigators); collaborators. CDC, Grants for Injury Control Research (Donald Marion, M.D., PI); Adenosine and TBI, NS38037, (8/2/01-7/31/02) $263,910, Patrick Kochanek, PI; iNOS and Traumatic Brain Injury, NS30318 (Patrick Kochanek, PI), Project 3 within the University of Pittsburgh Brain Trauma Research Center (BTRC), Donald Marion, PI. Protocol #3480500 (5/1/00-4/30/01), $11,215, Rachel Berger, PI, CHP GCRC. Oxidative Stress after Severe Head Injury in Infants and Children: Effect of Therapeutic Hypothermia, Laerdal Foundation, Hülya Bayır, PI.

B. Adenosine and TBI

Adenosine is produced during the breakdown of adenosine triphosphate (ATP) after TBI. Its powerful vasodilator, anti-excitotoxic, and anti-inflammatory effects may represent an important endogenous defense mechanism in injured brain. The role of adenosine as an endogenous neuroprotectant molecule, particularly early after TBI, and its potential participation in delayed cerebral swelling are being pursued both in the rat TBI model and in patients after TBI. We are beginning the 4th year of this RO-1-funded project. This project continues to be the most active area of research in Dr. Kochanek's laboratory this year and has produced a number of reports of studies in both patients and experimental models of brain injury. This work is being carried out in collaboration with Dr. Edwin Jackson in the Center for Clinical Pharmacology. In laboratory aspects of the research on this project, we have continued to evaluate the effect of local injection of adenosine receptor agonist and antagonists on cerebral blood flow. That work is carried out in collaboration with Dr. Chien Ho and Kristy Hendrich at the Pittsburgh NMR Center. A recent study was presented at the 2001 meeting of the National Neurotrauma Society—and demonstrated that adenosine receptor agonist mediated cerebrovasodilatory effects are mediated by the A2a receptor and can increase cerebral blood flow in both the normal and traumatically injured rat brain. A number of outcome studies of adenosine agonists are ongoing in collaboration with Dr. C. Edward Dixon in our center using the controlled

cortical impact (CCI) model. Manu Varma, an undergraduate from the University of Michigan who worked on that project in our laboratory again this summer, was just informed that his manuscript on this work is accepted for publication in the journal *Brain Research*. Using our CCI model, we are currently studying the A2a-receptor knockout mouse, obtained from Dr. Jiang-Fan Chen at the Massachusetts General Hospital and the A1-receptor knockout mouse obtained from Dr. Jurgen Schnermann at the NIH to begin to unravel the role of specific adenosine receptors in the mechanisms of secondary damage and repair after experimental TBI. Key collaborators on the RO-1 are Drs. Edwin Jackson, C. Edward Dixon, Chien Ho, Steven Graham, Donald Marion, and Ms. Kristy Hendrich.

Support: NIH RO-1, Adenosine and Traumatic Brain Injury, ($1,593,730, 08/02/99-07/31/03, Patrick M. Kochanek, M.D., PI).

C. Role of Inducible Nitric Oxide Synthase (iNOS) in the Inflammatory Response after TBI

iNOS is induced by cytokines and NF-κB is suggested to play an important role in the pathophysiology of sepsis outside of the central nervous system. Both beneficial and detrimental actions of iNOS have been reported. Using both inhibitors of iNOS and knockout mice, Dr. Elizabeth Sinz (1996-97 Charles Schertz Fellow) reported a powerful endogenous neuroprotectant effect of iNOS in experimental TBI. This area of study is carried out as part of our funded project within the University of Pittsburgh Brain Trauma Research Center (BTRC) Program Project. In collaboration with Drs. Kagan and Timothy Billiar, Hülya Bayır has been studying protein nitration and nitrosylation after experimental TBI using iNOS knockout mice. Nitrosothiols may represent a nitric oxide reservoir and could play important roles in signal transduction, immunomodulation, vascular regulation, and neurotransmission.

Support: NIH 2P50 NS30318, iNOS and Traumatic Brain Injury, ($582,986), Patrick Kochanek, M.D., PI, Key Collaborators: Robert SB Clark, M.D., C. Edward Dixon, Ph.D., Timothy Billiar, M.D., Valerian Kagan, Ph.D., Larry Jenkins, Ph.D., Xiaopeng Zhang, Ph.D., Hong Qu Yan, M.D., and Timothy Carlos, M.D., collaborators.

D. Emergency Interventions after TBI: Effect on Secondary Damage

Studies in this area of investigation were funded, this year, by both the Laerdal Foundation and the Curagen Corporation. Dr. Kimberly Statler (one of our T32 fellows) has been the leading investigator on this work. Dr. Statler presented a surprising paper showing that moderate hypothermia, applied after experimental TBI, expands lesion volume at 72 hours after injury in rats anesthetized with the narcotic fentanyl. That work was presented at the National Neurotrauma Society meeting and is *in press* as a full manuscript in the journal *Critical Care Medicine*. In that study, Dr. Statler discovered that hypothermia after TBI produces an enhanced stress response—reflected by higher serum catecholamine levels—compared to the normothermic condition. These studies

are in contrast to the remarkable neuroprotection that others and we have consistently observed with hypothermia in rats anesthetized with isoflurane. The importance of this work lies in the fact that patients are sedated with narcotics after TBI. It may be that to maximize the potential benefit of therapeutic hypothermia after TBI, sedation must be optimized. To further understand the mechanism underlying the effect of hypothermia on experimental TBI, we are carrying out studies evaluating the effect of hypothermia on gene expression using our mouse model of controlled cortical impact. This work is being carried out in collaboration with Dr. Melvin Heyes at the Curagen Corporation, a leader in gene culling technology. In an initial report, two summer students, Becky Sullivan and Gilna Alce published an abstract of work in *Critical Care Medicine* showing a robust beneficial effect of the resuscitative application of transient, moderate hypothermia in this model. This, to our knowledge, is the first report of the beneficial effects of hypothermia in a mouse model—and sets the stage for studying the combined effects of hypothermia in genetically modified mice. Finally, Dr. Statler also published an invited review on this area of work in the *Journal of Neurotrauma* that was based on a plenary talk by Dr. Kochanek, entitled "The Simple Model Versus the Super Model: Translating Experimental TBI Research to the Bedside." We hope to also soon apply proteomics approaches to the study of hypothermia in TBI in collaboration with Dr. Larry Jenkins in our Center.

Support: Laerdal Foundation, MRI Assessment of cerebral blood flow and calcium accumulation after TBI in rats: Effect of isoflurane versus Fentanyl, ($7,500, 1/1/00 – 6/30/01, Kimberly Statler, PI). Training in Pediatric Neurointensive Care and Resuscitation Research, T32-HD40686, National Center for Rehabilitation Research, National Institute of Child Health and Development, Patrick Kochanek, PI 9/25/00-4/30/01.

E. Magnetic Resonance Imaging (MRI) Assessment of Experimental TBI in Rats

Contemporary and novel MRI methods are being used to characterize our injury model and facilitate the testing of novel therapies in experimental TBI in rats. The goal of this work is to use non-invasive NMR methods to access acute physiologic derangements early after injury and to couple these to assessment of functional outcome at more delayed times after TBI. MRI methods were used to augment investigation in our study of both adenosine and anesthetics in experimental TBI. We have just begun to expand this application this year to the study of our mouse model of experimental TBI with the help of Kevin Hutchins. Dr. Ho's outstanding multidisciplinary NMR center for biomedical research continues to be a key collaboration for our work in experimental TBI and we hope to begin to collaborate with Dr. Eric Aherns in the area of microimaging applied to our mouse TBI model.

Support: NIH-NINDS 2P50 NS3031809 A1, Rat/Surgery/Imaging Core C, ($470,095 over 5 years, Patrick Kochanek, M.D., PI, Chien Ho, Ph.D., Co-PI, Kristy Hendrich, Donald Williams, Ph.D., and Steven DeKosky, M.D., Co-investigators). NIH Grants RR-

03631 and RR-10962, (Chien Ho, PI) support the Multidisciplinary Pittsburgh NMR Center at Carnegie Mellon University. NIH PAR00-031, In-Vivo MR Microscopy Instrumentation at 11.7 Tesla ($500,000, Chien Ho, Ph.D., --submitted 3/13/00).

2. Studies directed by C. Edward Dixon, Ph.D.

Research Interests

Research in Dr. Dixon's laboratory is directed towards understanding the mechanisms of cognitive deficits following TBI. Current studies are evaluating the effects of brain injury on dopaminergic and cholinergic systems and the relationship between these changes and the induction and recovery cognitive deficits. Experimental neurotherapeutic studies are ongoing to evaluate the effects of neurotrophic growth factors and neurotransmitter receptor activation on recovery of function. Clinical studies include an ongoing randomized clinical trial of amantadine hydrochloride on neuropsychological measures of frontal lobe function and measuring CSF and extracellular levels of catecholamines and markers of oxidative injury in humans acutely after brain trauma.

A. Dopaminergic/Cholinergic Mechanisms of TBI

Recovery of cognitive function after TBI is a dynamic process in which alterations in neurotransmitter systems do not likely occur in isolation. During the previously funded period we have observed that substantial cholinergic neurotransmission deficits can occur without a chronic (4-week post injury) loss of cholinergic cell bodies. We also have extensive data that TBI causes chronic changes in key dopaminergic proteins that occur concomitantly with these cholinergic changes. Numerous studies have demonstrated that the dopaminergic innervation of medial septum and diagonal band of broca (medial septal area [MSA]) regions that are dense with cholinergic neurons, can affect hippocampal acetylcholine (ACh) release, especially via D1 receptor agonists. Furthermore, we have compelling preliminary data that dopaminergic innervation of cholinergic nuclei is reduced after TBI. For this project, we propose to logically extend our previous findings to hypothesize that cognitive deficits following TBI may be, at least partially, attributable to decreased dopamine (DA) modulation of septohippocampal cholinergic function. A systematic series of studies are proposed to test this hypothesis. For this project, we will focus on DA modulation of the selectively vulnerable septohippocampal cholinergic system. This provides us with a prototypical system to examine the effects of TBI on interactive neurotransmitter systems. To better grade an effect of TBI on these systems, we will compare in the MSA the effects of TBI to an established model of DA deafferentation effects; 6-hydroxydopamine (6-OHDA) -induced DA denervation. We will examine the effects of TBI and 6-OHDA lesions on DA modulated ACh release in the hippocampus and DA release in the medial septum. Dr. Dixon will also determine whether changes in hippocampal ACh release is associated with altered D1 receptors in the MSA using quantitative autoradiography, and DA-fiber/cholinergic neuron interactions using a tyrosine hydroxylase/choline acetyltransferase double-immunolabeling method following TBI. Dr Dixon's group will determine the effect of

exogeneous administration of neurotrophic factors that promote DA neuronal survival on DA biochemical markers, cognitive deficits, as well as hippocampal ACh release and MSA DA release following TBI. Lastly, Dr. Dixon will determine the effects of clinically relevant DA agonist therapies on cognitive deficits, as well as hippocampal ACh release and MSA DA release following TBI. Our long-term goal is to develop new therapies to accelerate cognitive recovery following TBI.

During this year, we have found that TBI can produce chronic changes in proteins necessary for DA neurotransmission. We have also found that TBI can produce a reduction in DA release in the medial septal region at 2-weeks postinjury and that the number of tyrosine hydoxylase (TH)-positive fibers with the medial septum and diagonal band are decreased after TBI. Immunohistochemical and Western blot studies have revealed a distributed upregulation of TH and downregulation of DAT protein levels. Western blot studies have found decreases in D2 receptor protein levels in the striatum at 4-wks postinjury. We have also demonstrated that DA agonists can enhance recovery of cognitive function after TBI. Overall, there is new evidence that ACh and DA systems are altered chronically after TBI. We also have preliminary data that markers of DA innervation of the septal region are chronically diminished after TBI.

Support: NIH-NINDS, Chronic Changes in Neurotransmission Following Traumatic Brain Injury, R01 NS-33150-06 ($1,000,000 / $484,819 over 5 years, 4/1/00-3/31/05, C. Edward Dixon, Ph.D., PI).

B. Functional Outcome Core

During this year, the Functional Outcome Core has evaluated post-injury function in several hundred rats and mice for seven different Principal Investigators associated with the Safar Center.

The Functional Outcome Laboratory Core Facility provides a centralized site and highly standardized procedural control for all animal experiments employing functional outcome as an endpoint following brain injury to rats. The Functional Outcome Laboratory Core gives the investigators of the University of Pittsburgh Brain Trauma Center the capability to assess the effects of physiological manipulations and therapeutic interventions of recovery of function after experimental brain injury.

Support: NIH, BTRC Supplement—Functional Core to P50 NS-30318-041A ($274,583 over 4 years, 4/1/96-3/31/00, C. Edward Dixon, Ph.D., PI).

C. Oxidative Mechanisms of Severe TBI: Relationship to Chronic Frontal Lobe Cognitive Deficits.

The primary goal of this project is to test the hypothesis that TBI will produce an increase in DA which autoxidizes to generate hydroxyl and free radicals that produce oxidative damage to proteins in frontal lobe regions, and these changes are predictive of long-term

performance on neuropsychological measures of frontal lobe function. We will test this hypothesis by utilizing in vivo microdialysis to determine whether extracellular levels of markers of catecholamine protein oxidation, nitration markers, and catecholamine levels predict 6-month neuropsychological outcome on measures of frontal lobe function in humans after severe TBI.

The University of Pittsburgh BTRC has a productive infrastructure to conduct mechanistic studies of available human extracellular fluid, and CSF specimens collected during the acute phase of TBI. The funds provided by this grant are being used to provide the additional support necessary for this existing multidisciplinary team to collect extracellular and CSF samples for analysis of DA and behavioral outcome in TBI patients. If a link is established between DA oxidation and frontal lobe behavioral deficits after severe TBI in humans, than our long-term goal will be to clinically evaluate the effects of therapies that reduce catecholamine-mediated oxidative brain injury on behavior outcome. Our ultimate objective is to reduce disability following TBI and consequently the cost of TBI to society.

Support: The Pittsburgh Foundation – The John F. and Nancy A. Emmerling Fund, Oxidative Mechanisms of Severe Traumatic Brain Injury: Relationship to Chronic Frontal Lobe Cognitive Deficits, reference #: M1999-0080, ($95,958 over 3 years – no indirect costs, 07/01/99-06/30/02, C. Edward Dixon, PI).

D. Examination of the Cellular Mechanisms of Mesocortical Dopaminergic Deficits after TBI in a Rodent Model Using Biochemical Indices of DA Autoxidation and Biochemical, Molecular Biological and Immunohistochemical Indices of DA Metabolism and Neurotransmission.

The goal of this project is to examine the cellular mechanisms of mesocortical dopaminergic deficits after TBI in a rodent model using biochemical indices of DA autoxidation and biochemical, molecular biological and immunohistochemical indices of DA metabolism and neurotransmission. Neurochemical and immunohistochemical markers of DA neurotransmission in the dopaminergic ventral tegmental/forebrain systems, as well as functional deficits, will e assessed at specific time points following injury suggested by our preliminary data. The effects of therapies that either reduce oxidative damage of DA terminals and/or chronically stimulate DA activity on neurochemical and immunohistologic markers, and on functional performance will be assessed following TBI. Lastly, the relationship between early biochemical markers of DA activity to neuropsychological outcome measures specific to frontal lobe function will be evaluated in severe TBI patients. This project represents the first systematic examination of the mechanisms of induction and recovery of catecholaminergic cognitive deficits after TBI. Our long-term goal is to develop new therapies to attenuate the induction and enhance the recovery of DA-mediated neurobehavioral deficits after TBI.

Support: NIH-NINDS, Mechanisms of Prefrontal Dysfunction Following Brain Trauma, R01 NS-40125-01 ($800,000 / $376,775 over 4 years, 3/1/00-3/31/04, C. Edward Dixon, Ph.D., PI).

E. Effects of Amantadine Hydrochloride on Functional Outcome after TBI: A Randomized, Multi-center, Placebo-Controlled, Clinical Trial.

This project will study one hundred patients with TBI. These will include patients admitted to participating regional rehabilitation hospitals following the acute phase, post-injury, as well as chronic TBI patients recruited through the Neurobehavioral clinic of a major urban academic medical center. Efficacy of drug treatment will be assessed with a double-blind, cross-over design. At each study site, the patients will be randomized into an initial amantadine hydrochloride (AMH) or a placebo group, treated for 3 months, then crossed over to the other treatment for an additional 3 months. A set of measures of each patient's functional status (including the Neurobehavioral Rating Scale, the Glasgow Outcome Scale, and the Disability Rating Scale) will be administered at the time of their admission to the study (baseline), three months after initiation of the intervention, and 3 months after the opposing treatment. Additionally, neuropsychological tests specific to memory and frontal lobe function will also be administered at the same time points. These assessment data will provide the requisite dependent measures to evaluate the following specific hypotheses involving the AMH and placebo patient groups.

Support: CDC, CIRCL - Acute Care Project 1, R49 CCR-312296 ($151,000 / $45,000 year 2, 9/01/98–8/31/02, C. Edward Dixon, PhD., PI).

3. Studies by Robert S. B. Clark, M.D.

A. Endogenous Neuroprotectant Gene Expression after TBI

This research focuses on the genetic regulation and execution of delayed neuronal death in selectively vulnerable neurons after TBI. We have now characterized the expression of several potential cell death-suppressor genes and their translated proteins including bcl-2 gene family members and heat shock protein 72 (endogenous neuroprotectants), as well as potential cell death-effector genes including the pro-apoptotic bcl-2 gene family member bax. These genes appear to be upregulated and/or activated after TBI in both our experimental model (CCI injury with secondary hypoxemic insult followed by resuscitation in rats) and in humans. Studies documenting that bcl-2 family genes may be important in both adult and pediatric patients after head injury were reported previously in the *FASEB Journal* and the *Journal of Pediatrics*, respectively. A role for heat shock proteins after human head injury is also being investigated. More recent studies have suggested that regulation of some of these proteins is via post-translational modification, including the bcl-2 family members bad and bag-1. Bag-1 regulates the chaperone function of heat shock proteins, pointing to a direct interaction between these two classes of endogenous neuroprotectants. A report by pediatric CCM fellow, Neal Seidberg,

entitled "Alterations in inducible 72-kDa heat shock protein and the chaperone cofactor BAG-1 in human brain after head injury" is in press in the *Journal of Neurochemistry*. Future studies aim to determine to what extent these genes and their translated proteins contribute to the regulation and execution of neuronal death by using novel molecular (antisense oligonucleotides and viral transgenes) and pharmacologic (fusion protein-coupled heat shock protein) therapies in our experimental models, to provide support for future clinical trials using similar strategies. Finally, in studies addressing another endogenous neuroprotectant pathway, Dr. Xiaopeng Zhang presented a paper at the National Neurotrauma Society Meeting entitled "Increase in phosphorylated protein kinase substrates after human head injury."

B. Caspase-Mediated Neuronal Death after Head Injury

Increasing evidence suggests that activation of caspases regulate and execute programmed cell death after TBI in experimental models and in humans. Accordingly, the objective of this research is to develop pharmacological and molecular treatment strategies that reduce caspase-mediated programmed-cell death after TBI. Last year, we described the expression and activity of caspase-3 in our experimental model of TBI in the *Journal of Neurochemistry*. Importantly, a caspase-3 inhibitor administered after trauma reduced cell death; although, no effects in behavioral outcome could be demonstrated. Potential roles for caspase-1 and -3 were also described in the above-mentioned paper published in the *FASEB Journal*. Studies examining other more potent caspase inhibitors, and combination treatment strategies targeting multiple points in the programmed cell death cascade are ongoing.

C. Divergent Pathways of Cell Death after Brain Injury

It is clear that both apoptotic and necrotic cell death contribute to neuronal cell loss after acute brain injury; however, recent data suggest that this is in fact over simplistic, and that multiple, interrelated pathways exist. A key regulator in this regard is the mitochondrial protein AIF. Work by Dr. Xiaopeng Zhang has clearly demonstrated that AIF-mediated cell death occurs after experimental TBI. That work was presented by Dr. Zhang at the 2001 Meeting of the Society for Neuroscience. This process also appears operative in neurons in vitro as work by Dr. Zhang in concert with Dr. Lina Du has shown that peroxynitrite-induced injury in neurons is associated with nuclear translocation of AIF, large-scale DNA fragmentation, and cell death. This cell death is inhibited using a peroxynitrite decomposition catalyst or PARP inhibitors. These data were combined and published recently by Dr. Zhang et al., as a full manuscript entitled "Intranuclear localization of apoptosis-inducing factor and large scale DNA fragmentation after TBI in rats and in neuronal cultures exposed to peroxynitrite" in the *Journal of Neurochemistry*. Further work intends to tease out the contribution of these divergent pathways of cell death using multiple strategies in collaboration with Drs. Jun Chen, Steven Graham, Patrick Kochanek, Csaba Szabo (Inotek Corp., Beverly, MA), Simon Watkins, Hector Wong (Cincinnati Children's Medical Center), Ian Reynolds, and Donald Marion.

D. PARP Activation after TBI

The study of PARP in experimental TBI is an expanding area of investigation at our center. PARP is an abundant nuclear enzyme with a role in DNA repair pathways. However, in the setting of energy failure, it is suggested that excessive ADP-ribosylation of proteins resulting from activation of PARP leads to marked nicotine adenine dinucleotide (NAD) depletion and exacerbation of energy failure. Drs. Whalen, Clark, and Kochanek collaborated with Dr. Csaba Szabo (an expert in the area of PARP and sepsis at the Inotek corporation) to study the PARP knockout mouse in our model of experimental TBI. We previously reported highly significant level of protection against functional deficits after TBI in PARP knockout vs wild-type mice. This effect was the greatest beneficial effect that we have observed with any agent in the CCI model of TBI. That work was published as a rapid communication in the *Journal of Cerebral Blood Flow and Metabolism*. Dr. Margaret Satchell, a 4th year pediatric CCM fellow who is working on our T-32 grant from NICHD, has aggressively pursued this promising mechanism in collaboration with Dr. Szabo using novel inhibitors of PARP activation in our TBI model. Abstracts of that work were presented last year as described in the 2000-2001 annual report. Dr. Satchell has pursued clinical studies of PARP activation and nitrosative stress after TBI in adults. She presented this work recently at the 31st SCCM Critical Care Congress and received an Annual Scientific Award from the Society of Critical Care Medicine. She previously received the Murray Goldstein Award from the Neurotrauma Society for her work. This exciting project, with Dr. Clark as PI, is funded within the University of Pittsburgh BTRC Program Project Grant application (Donald Marion, PI).

Support: RO1-NS38620-03, Caspase-Mediated Neuronal Death After Head Injury ($584,022 total direct costs over 4 years beginning 2/1/99, Robert Clark, M.D., P.I.); KO8-NS01946-05, Role of Neuroprotective Genes After Traumatic Brain Injury ($455,960 total direct costs over 5 years beginning12/1/96, Robert Clark, M.D., PI; Steven Graham, M.D., Ph.D. and Patrick Kochanek, M.D., Sponsors); P01-NS30318, PARP Activation After TBI, Project 4 of the BTRC Program Project ($595,000 total direct costs over 5 years beginning 6/1/00, Robert Clark, M.D., P.I.).

4. Studies by P. David Adelson, M.D.

A. Severe TBI in Immature Rats

Diffuse injury and cerebral swelling are important components of the clinical picture in children with severe TBI. Unfortunately, there has been only limited investigation of pediatric TBI in laboratory models, and specific treatments are lacking. The major goal of this program is to define the pathophysiologic response to severe TBI in the immature rat and to better understand the mechanisms involved with neural injury and recovery.

This year, support for this area of research has been obtained from the multiple sources including the NIH, Copeland Foundation, Betty Freiberg Fund, and the University of Pittsburgh. Studies being carried out in Dr. Adelson's laboratory include focal and diffuse experimental TBI. Characterization of the long-term functional and histopathological consequences of severe diffuse and focal TBI was performed, in collaboration with Drs. Dixon and Jenkins. This work has been presented at the annual meetings of the National Neurotrauma Society. Remarkably, sustained functional deficits were observed out to three months following diffuse injury. This indicates that the diffuse injury model, unlike fluid percussion or CCI, produces enduring functional deficits in the immature rat. Other, collaborations with Drs. Whalen, Jenkins, and Kochanek, include the histopathologic response to TBI of both the CCI and the diffuse injury models in the immature rat. There were striking differences in the injuries incurred and in the expression of adhesion molecules and leukocyte influx in these models. Acute inflammatory response was observed only in the area of contusion produced by CCI. This, to our knowledge, is the first direct comparison of the inflammatory response in focal and diffuse injury models, and suggests that the deficits produced by diffuse injury are manifest despite a paucity of acute inflammation. These comparisons between focal and diffuse will be the mainstay of the laboratory over the next few years. In collaboration with Dr. Chien Ho, Kristy Hendrich and the talented group at the Pittsburgh NMR Center at Carnegie Mellon University, the time course of changes in blood flow and brain swelling are being identified using non-invasive MRI methods in the diffuse and focal injury models. Lastly, additional areas of study include the testing of novel therapies in the focal and diffuse injury models of TBI in the immature, including moderate hypothermia, excitotoxic blockade and cyclosporin A as well as others to be introduced after further defining mechanistic etiologies of damage.

Support: KO-8NS01809-01A1, Severe Diffuse Traumatic Brain Injury in Immature Rats ($415,260 over 5 years beginning 12/96, P. David Adelson, M.D., PI, Patrick Kochanek, M.D.)

B. Hypothermia for Severe TBI in Children

The major goal of this project is to test the safety and efficacy of hypothermia in children after severe head injury. This program has been funded at an R01 level by the NIH and seeks to investigate hypothermia as a treatment of TBI in children, with a special emphasis on the development of novel methods for initial and outcome assessment. Dr. Adelson is the principal investigator of this multicenter study that includes 8 centers. Dr. Harvey Levin at the Baylor College of Medicine, is a key co-investigator on that important proposal. In addition, in collaboration with Drs. Kochanek, DeKosky, and Graham and others further ongoing research includes the effect of therapeutic hypothermia on both excitotoxic and inflammatory markers of brain injury in infants and children, the effect of severe TBI on language and speech acquisition and recovery, cerebral blood flow and metabolism after injury, long-term effects of mild-moderate head injury, and other collaborative and related efforts.

Support: KO-8 NS01809-01A1, Severe Diffuse Traumatic Brain Injury in Immature Rats ($415,260 over 5 years beginning 12/96, P. David Adelson, M.D., PI, Patrick Kochanek, M.D., Sponsor); Pittsburgh Foundation, Functional and Cerebrovascular Response to Severe Diffuse Traumatic Brain Injury in Immature Rats ($50,000 over 2 years beginning 7/96, P. David Adelson, M.D., PI); Competitive Medical Research Fund, Functional and Cerebrovascular Response to Diffuse Traumatic Brain Injury in Immature Rats ($25,000 over 1 year beginning 8/97, P. David Adelson, M.D., PI).

5. Studies by Steven DeKosky, M.D.

A. Neurotrophic Response to TBI

Dr. DeKosky's laboratory studies assessing the role of neural cells and their products in the brain's attempt at repair following TBI. Our laboratory is particularly interested in the cytokine and anti-oxidant cascade following injury, and the role of these inflammatory proteins in the upregulation of neuroprotective proteins such as nerve growth factor (NGF). Our goal is to elucidate the brain's injury response and provide insight into possible therapeutic interventions.

We have examined several inflammatory and anti-oxidant molecules following injury. One important protective response to injury is the upregulation of antioxidant proteins such as catalase and glutathione peroxidase. Our data show that catalase (CAT) and glutathione peroxidase (GPx) increase in the lesioned cortex after TBI, peaking at 3 and 7 days, respectively. In the hippocampus, CAT and GPx increase at 1 day, peaking at 7 days, while superoxide dismutase (SOD) levels actually decrease by 6 hours post-trauma. Hypothermia treatment attenuates the rise in GPx, while increasing activity of SOD above sham levels. We previously had shown that reduction of IL-1 after TBI inhibits NGF expression and suppresses antioxidant upregulation. Subsequent experiments have examined whether p75 and trk A, the receptors for NGF, are elevated following TBI. Our analyses suggest that TBI elevates the mRNA and protein for both p75 and trk A in the cortex and hippocampus. These major studies are of interest to our laboratory because, in addition to furthering understanding of the biochemical changes following TBI, they may provide insight into human neurodegenerative diseases, including Alzheimer's disease.

B. Effects of TBI on Amyloid Precursor Protein (APP) Metabolism

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by neuronal loss in discrete brain regions and by formation of neurofibrillary tangles and beta-amyloid associated neuritic plaques. A major component of these plaques is the 42-43 amino acid amyloid beta (Aβ) peptide that is cleaved from the transmembrane region of amyloid precursor protein (APP).

One of the known risk factors for AD is TBI. Therefore, alterations in APP processing may play an important role in the pathogenesis of both TBI and AD. We are examining the factors involved in regulating the expression of APP after TBI in rats. Preliminary

data demonstrate a trauma-induced increase in APP and Aβ levels. Determining the factors and conditions that regulate APP metabolism will provide information for the role of this compound in TBI and for the development of therapeutic strategies for AD. We are currently developing treatment strategies that will enable manipulation of APP processing after TBI, with an eye toward suppressing Aβ, which produces apoptosis and oxidative stress after injury and in AD. Information gained by these studies will establish the therapeutic importance of manipulating APP metabolism, and offer new opportunities for suppressing the pathological deposition of Aβ in AD.

To better understand the relationship between TBI and AD, we are investigating the expression of AD markers, including amyloid, in surgically resected tissue samples and CSF from head-injured patients. Our data demonstrate that amyloid-related pathological and biochemical changes after TBI are similar to those observed in AD. Shortly after head injury, there is increased production of APP. In addition, the two Aβ species (and particularly the "early" Aβ42) are increasingly being deposited in the tissue, while they are depleted in the CSF. In contrast to these rapid changes in amyloid metabolism, neurofibrillary tangles, another major pathological hallmark of AD, are not found in these patients. Our present results suggest that Aβ(42) may play a critical role in plaque formation after brain injury, similar to findings in AD brains. Studies are underway to further characterize these and other changes that support common pathological mechanisms in TBI and AD. Because the normal biological function of APP is unknown, determining the factors and conditions that regulate APP metabolism will provide information for the development of therapeutic strategies for both TBI and AD.

Support: Core C of 2 P50 NS30318-04A21, Project #3 in the University of Pittsburgh Head Injury Research Center (Steven DeKosky, M.D., PI).

6. Studies by Steven Graham, M.D., Ph.D.

A. Excitotoxicity and Programmed Cell Death

Dr. Graham's laboratory studies the molecular and cellular mechanisms of neuronal cell death. In collaboration with the Safar Center, Dr. Graham's laboratory investigates neuronal death in TBI. Ongoing studies concern the role of excitatory amino acids and free radicals in pathogenesis of brain injury. The recent emphasis of the laboratory has been the genetic mechanisms that regulate neuronal cell death. In particular, the role of genes that regulate programmed cell death, the bcl-2 and the cysteine protease family of genes, is being investigated in trauma.

Cyclooxygenase-2 (COX2), the inducible isoform of the enzyme that catalyzes the formation of prostaglandins is also being investigated. Expression of COX2 is induced by neuronal excitation and COX2 activity produces free radicals, so COX2 may be an important mechanism whereby excitotoxicity is expressed.

Support: Core C of 2 P50 NS30318-04A21, Project #1 in the University of Pittsburgh Head Injury Research Center (Steven Graham, M.D., Ph.D., PI). Department of Veterans Affairs/Department of Defense Brain Trauma Initiative - Merit Review (Steven H. Graham, M.D., Ph.D., PI, 4/1/95 - 3/31/98, 20% of VA Time. Department of Veterans Affairs, The Role of Inducible Cyclooxygenase in Delayed Neuronal Death. Current Year Direct Costs: $143,000. Technicians: Ann Stetler, Marie Rose, Post doc: Li Zhu, Ph.D.).

7. Studies directed by Larry W. Jenkins, Ph.D.

A. Protein Synthesis, Memory and Pediatric Brain Injury

We have further examined the potential role of impaired protein synthesis in memory deficits after experimental pediatric TBI. There is extensive data suggesting that protein synthesis is critical for the consolidation of hippocampal dependent learning and memory. Protein synthesis is involved in developmental synapse formation, long-term potentiation (LTP) and during memory consolidation. Our initial study employing 2-D gel electrophoresis to examine global protein expression during the consolidation of spatial memory acquisition has just been submitted for publication. Proteomic studies have significant potential to expand our understanding of neural injury and therapy but have yet to be applied to TBI. The purpose of the present study was to examine global hippocampal protein changes in 17 PND rats 24 hrs after moderate CCI. Analysis was limited to a wide pH range (nonlinear pH 3-10) for isoelectric focusing with immobilized pH gradients (IPG strips) and large format (22 x 22 mm) SDS slab gels. We evaluated only the most soluble cellular protein fraction using hippocampal tissue protein lysates from six paired sham and injured rats. Approximately 1500 proteins spots were found in each gel with 40% spot matching of proteins. Of these 600 matched proteins 50% showed either a 2 fold increase or decrease, 20%, a 5 fold increase or decrease, and 10%, a 10 fold decrease or increase. Limited spot matching with existing protein databases showed changes in some important cytoskeletal (actin and tubulin), and cell signaling (phosphatidylinositol transfer protein and superoxide dismutase) proteins suggesting that this approach is both feasible and informative in the study of protein changes after pediatric TBI.

Our long-term goals are to also characterize some of the most important changes in neuronal signaling known to influence cognitive dysfunction after injury and determine if these changes can be normalized by delayed treatment with trophic factors Protein synthesis may be altered after TBI by changes in the phosphorylation state of PKB. Phosphorylated PKB (p-PKB) activation alters protein synthesis by phosphorylating the target of rapamycin protein kinase (mTOR/FRAP) that in turn phosphorylates 4EBP (p-4EBP) the repressor binding protein (4EBP) of eukaroytic initiation factor 4E (eIF4E). p-PKB also activates eukaroytic initiation factor 2α(eIF2α indirectly by phosphorylating glycogen synthase kinase 3 (GSK-3) reducing the phosphorylation of eIF2α and activating p-eIF2α. Thus, PKB phosphorylation modulates the selection of translated mRNA by eIF4E activity and the global rate of protein synthesis by increasing p-eIF2α activity. We

evaluated the level and distribution of brain p-PKB, p-4EBP, p-eIF4E, and p-eIF2α activity in injured or sham 17 PND rats at 6, 24 or 72 hr after moderate CCI using immunohistochemistry (n=5/group). TBI increased the levels of all impacted hippocampal p-proteins at only 6 hr except p-eIF4E suggesting an early but unsustained upregulation of PKB linked protein synthesis activators after pediatric CCI. We are further expanding these studies.

Support: NIH-NINDS, Protein Synthesis, Memory and Pediatric Brain Injury, R21 NS-40049, ($186,250/yr, 5/1/00-4/30/03, Larry W. Jenkins, Ph.D., PI).

B. Hypothermia and TBI

The overall goals of this project are to examine the effects of mild hypothermia treatment on secondary ischemic injury and neurotransmitter signal transduction after TBI. This project combines behavioral and structural morbidity studies with molecular pharmacology to examine some potential mechanisms of increased sensitivity of the posttraumatic brain to secondary cerebral ischemia and potential actions of hypothermic treatment on altered signal transduction after TBI.

Normally, secondary forebrain ischemia following mild TBI results in increased bilateral delayed CA1 neuronal death by 7 days after injury. During the last year we examined the use of a general serine/threonine protein kinase inhibitor staurosporine (10 ng) microinjected into the hippocampal CA1 sector before mild TBI and then examined CA1 neuronal death following mild TBI and combined secondary cerebral ischemia. Significant neuronal protection was found in the CA1 sector at 7 days of survival demonstrating that aberrant protein kinase activation may enhance posttraumatic ischemic sensitivity after even mild TBI. These studies are being prepared for publication submission.

We studied the effect of serum hyperglycemia on secondary ischemic brain injury after TBI. Serum hyperglycemia increases secondary ischemic brain damage after TBI; however, it is unknown if the traumatized brain is hypersensitive to the hyperglycemic exacerbation of secondary ischemic injury. We examined if posttraumatic serum hyperglycemia at levels of 400-mg% decreases the threshold for secondary ischemic injury after TBI. Normoglycemic rats receiving mild TBI and 6 min of forebrain ischemia also had CA1 neuronal death after 7-day survival. In contrast, hyperglycemic rats subjected to mild TBI and 6 min of forebrain ischemia developed seizures leading to status epilepticus within 18-24 hr after injury and had extensive neuropathological damage in many brain regions in proportion to seizure activity duration. These data suggest that mild TBI predisposes the traumatized brain to sustain more damage to hyperglycemia related exacerbation of ischemic injury compared to the non-traumatized brain by increasing parenchymal sensitivity to secondary ischemic damage in multiple regions. This manuscript is being prepared for publication.

We also published a comparison CA1 hippocampal CBF profiles before, during and after 6 min of forebrain ischemia in normal or mildly hypothermic rats using laser Doppler flowmetry for the first time. During the ischemic insult there were intergroup differences in the magnitude of CBF decreases in the CA1 region. In both normo- and hypothermic groups, CBF returned to preischemic values within one minute of reperfusion but hypothermic rats had more sustained hyperemia. Hypothermic rats also had a quicker EEG recovery and less delayed CA1 neuronal death. These data suggest ischemic blood flow to the CA1 sector was increased by intraischemic mild hypothermia, which may contribute to the greater benefit of intraischemic hypothermic neuroprotection as compared to immediate postischemic hypothermia treatment. This study was published by *Brain Research.*

These collective studies suggest that hypothermia may provide benefit to head injured patients by modifying signal transduction before posttraumatic secondary ischemia or delayed neuronal death after secondary ischemia. The suspected mechanism is by preserving more normal serine/threonine protein kinase function.

Support: NIH-NINDS, Hypothermia and Trauma, R01 NS-35365, ($1,177,841 total award, 5/1/96-4/30/02, Larry W. Jenkins, Ph.D., PI).

8. Studies directed by Anthony E. Kline, Ph.D.

TBI affects 1.5 to 2 million people in the US each year, making it one of the more prevalent and debilitating of all neurological disorders. Approximately 300,000 of the TBI cases are severe enough to warrant hospitalization, where 50,000 die. Of the 250,000 survivors, 100,000 endure long-term disabilities that require rigorous, lengthy, and costly medical and rehabilitative care. In addition to the medical expenses associated with TBI, societal costs are also significant in terms of loss wages due to the inability to resume employment. While the true cost of TBI is incalculable, it is estimated at $100,000 annually per patient or about $48.3 billion per year. TBI is a serious and survivable medical problem with no acknowledged treatment. Therefore, empirical investigation of therapeutic strategies that may facilitate the recovery process after TBI, such as those directed by Dr. Kline and colleagues at the Safar Center and department of Physical Medicine & Rehabilitation (PMR) are essential. Equally important is the identification of pharmacological agents that may be detrimental to functional recovery after TBI.

A. Protective Effects of Serotonin$_{1a}$ (5-HT$_{1A}$) Receptor Agonists Against TBI-Induced Cognitive Deficits and Histopathology

Serotonergic pathways originating in the raphe nuclei have extensive projections to brain areas involved in cognition and 5-HT receptor agonists and antagonists alter these processes. Of all the 5-HT receptors characterized thus far, the 5-HT$_{1A}$ is the most widely studied. 5-HT$_{1A}$ receptors are abundantly expressed in brain regions, such as the cortex and hippocampus, that play key roles in learning and memory and that are susceptible to

neuronal damage by TBI. Several studies have reported decreased histopathology after focal or global cerebral ischemia in both rats and mice treated with 5-HT_{1A} receptor agonists. Because of the beneficial effects in these model systems, we examined them in our CCI model of TBI that produces many of the characteristics of human brain injury. The high affinity 5-HT_{1A} receptor agonist Repinotan HCL (BAY x 3702) was given (i.v.) as a 4-hr continuous infusion commencing 5-min after TBI or sham injury. Rats were evaluated for spatial learning in the MWM on post-operative days 14–20 and examined histologically at four weeks after TBI. Repinotan significantly attenuated spatial learning deficits as demonstrated by decreased latencies to locate a submerged (hidden) platform in a water maze task compared to the injured vehicle-treated group. Repinotan also attenuated histopathology as evidenced by significantly more hippocampal CA_1/CA_3 neurons and smaller cortical lesion volumes vs. the vehicle group. This study was the first to demonstrate beneficial effects with a 5-HT_{1A} receptor agonist in any model of TBI and is published in the journal *Neuroscience* (2001).

The positive demonstration of marked neuroprotection and cognitive improvement after acute Repinotan HCL treatment was the impetus for further investigation of 5-HT_{1A} receptor agonist treatments after TBI. In a follow-up study, we investigated whether 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) would produce similar beneficial effects. Using our standard injury and assessment paradigms, we found that 8-OH-DPAT exhibited significantly reduced latencies in locating the hidden platform vs. the vehicle group over time, which is indicative of improved learning and memory. Additionally, significantly more CA_3 surviving neurons were observed in the 8-OH-DPAT group relative to the vehicle group. This study is published in the journal *Neuroscience Letters* (2002). The beneficial effect on functional and histological outcome with the 5-HT_{1A} receptor agonist 8-OH-DPAT, coupled with our significant Repinotan HCL data, lend support for continued investigation of this novel therapeutic strategy. Collaborators include C. Edward Dixon, Ph.D., Jaime Massucci, B.S., and Jianyun Yu, M.D. from the Department of Neurological Surgery and Safar Center and Ross Zafonte from the Department of PMR.

B. The Effect of the Anti-Inflammatory Cytokine, Interleukin-10, Coupled with Moderate Hypothermia after TBI

TBI produces long-term disturbances in neurobehavioral and cognitive function. One mechanism for the detrimental effects may be the initiation of inflammatory processes such as the synthesis of proinflammatory cytokines that are implicated in secondary tissue damage. Experimental and clinical studies of TBI have shown robust inflammatory responses, including the early production of cytokines and the upregulation of (E)-selectin and intercellular adhesion molecule-1 (ICAM-1) on cerebrovascular endothelial cells. Previous studies using the CCI or fluid percussion (FP) injury models of TBI have shown that neutrophils accumulate in the brain as early as 4 hr after injury and reach peak levels by 24-72 hrs. Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that inhibits a variety of macrophage responses including the synthesis of cytokines, adhesion molecules, and chemokines. Moreover, hypothermia may also attenuate TBI-induced

inflammatory responses. Hypothermia has already been shown to benefit outcome after TBI. Improved functional and/or histological outcomes have been demonstrated following FP, weight drop, and CCI injury. In humans, significant reductions in intracranial pressure, as well as improved outcome have been shown with moderate hypothermia. Thus, we sought to evaluate the effects of IL-10 coupled with hypothermia on functional and histological outcome after experimental TBI. Briefly, fifty isoflurane-anesthetized rats underwent a CCI or sham injury and then were randomly assigned to one of five conditions (TBI/Vehicle Normothermia (37° C), TBI/Vehicle Hypothermia (32° C for 3 hr), TBI/IL-10 Normothermia, TBI/IL-10 Hypothermia, and Sham/Vehicle Normothermia). Human IL-10 (5μg) or vehicle was administered (i.p.) 30 min after surgery. Function was assessed by established motor and cognitive tests on post-operative days 1-5 and 14-18, respectively. Cortical lesion volume and hippocampal CA_1/CA_3 cell survival were quantified at 4 weeks. Brain sections from 15 additional rats were immunohistochemically assessed (MoAB RP-3) to determine neutrophil accumulation at 5 hrs after TBI. The administration of IL-10 after TBI produced an ~75% reduction in the number of RP-3-positive cells in both the normothermic and hypothermic groups vs. the normothermic vehicle-treated group ($P< 0.05$), but did not improve functional outcome. In contrast, hypothermia alone enhanced both motor and cognitive function and increased CA_3 neuronal survival after TBI. Contrary to our hypothesis, systemic administration of IL-10 combined with hypothermia did not provide synergistic neuroprotective effects after TBI. Rather, IL-10 administration suppressed the beneficial effects produced by hypothermia alone after TBI. Anti-inflammatory therapies can represent a dual-edged sword, exhibiting both beneficial and detrimental effects. Since hypothermia inhibits inflammatory responses – along with a variety of other mechanisms – after injury, combination with additional anti-inflammatory therapies may not be warranted. This study was published in the journal *Brain Research* (2002). Collaborators include Bryan Bolinger, B.S., C. Edward Dixon, Ph.D., Patrick Kochanek, M.D., Timothy Carlos, M.D., Hong Yan, M.D., Larry Jenkins, Ph.D., and Donald Marion, M.D.

C. Role of Environmental Enrichment (EE) after TBI

Enriched housing, which provides a complex, stimulatory, and social environment, and may be considered a rodent correlate of physiotherapeutic intervention, has been extensively studied in numerous experimental conditions. EE has been reported to increase brain weight, dendritic arborization, synaptogenesis, and to decrease apoptosis of neuronal precursor cells in the hippocampal dentate gyrus. Rats housed in EE for 30 days exhibit significantly higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus than rats housed in standard conditions. EE has also been shown to increase the expression of brain-derived neurotrophic factor mRNA in the rodent hippocampus. Furthermore, EE has been shown to improve spatial memory and reduce contusion lesion volume. EE has also been demonstrated to improve motor performance on a beam walk task or sensory neglect after cortical lesions. In or laboratory we are comparing the effect of 28 days of EE with standard living conditions on functional and histological outcome after TBI. The data suggest that EE is superior to standard housing

in facilitating functional recovery and suggests that this interventional strategy may be useful in a rehabilitative setting by augmenting pharmacotherapies. On-going studies in our laboratory are examining the role of EE coupled with the 5-HT_{1A} receptor agonists 8-OH-DPAT and buspirone on neurobehavioral and histological outcome after TBI. Collaborators include Amy Wagner, M.D., C. Edward Dixon, Ph.D., and Ross Zafonte, D.O. An RO1 grant entitled "Serotonergic and Environmental Therapies for TBI" has been submitted to further examine the relationship between EE and 5-HT_{1A} receptor agonists on the recovery process after TBI.

D. Effects of Atypical Antipsychotics on Functional Outcome after TBI

Over 1 million survivors of TBI receive maintenance pharmacotherapy, of which a substantial number receive antipsychotic agents for the treatment of psychoses, agitation and aggression, and other maladaptive behaviors. The incidence of agitation after severe TBI varies from 11% to 50%. In spite of the common clinical use of antipsychotics, the motor and cognitive risks vs. benefits are unclear. Early experimental studies by Feeney and colleagues have shown that the administration of antipsychotics (e.g., haloperidol) retard functional recovery after TBI. Moreover, the administration of such agents reinstates deficits in subjects appearing to be "recovered." Recently, Goldstein and colleagues have shown similar detrimental effects on motor function with haloperidol and clozapine after ablation-induced brain injury. Our laboratory is currently evaluating the effects of single (24 hr after TBI or sham injury) and/or chronic (24 hr – 28 days) administrations of the atypical antipsychotic risperidone on motor (beam-balance and beam-walk) and cognitive (spatial learning and memory) functioning in rats after TBI. Additionally, risperidone is being compared to the classical antipsychotic, haloperidol. The results from these studies should provide a clearer understanding of the effects of antipsychotic treatments in the recovering brain. These studies are being conducted in collaboration with Drs. Ross Zafonte and C. Edward Dixon.

Support: NIH-NICHD R03 HD043851-01, Interaction of serotonin and cholinergic systems after TBI, $144,233 for two years (04/01/03 – 03/31/05). Anthony E. Kline, Ph.D., PI. The Pittsburgh Foundation, Evaluation of the serotonergic$_{1A}$ receptor agonist, 8-OH-DPAT, on biochemical, functional, and histological outcome following traumatic brain injury in rats, $19,096 for one year (2001-2002). Anthony E. Kline, Ph.D., PI. The Pittsburgh Foundation, Efficacy of interleukin-10 (IL-10) coupled with moderate hypothermia in neurobehavioral, cognitive, and histological outcome following traumatic brain injury in rats, $24,261 for one year (2000-2001). Anthony E. Kline, Ph.D., PI.

Studies Conducted by Amy K. Wagner, M.D.

1. Clinical Gender Differences in TBI Pathophysiology

There is conflicting evidence as to whether there are gender differences with TBI pathophysiology and outcomes. Some clinical studies have reported that, even after

adjusting for injury severity women often fare worse. Previous work by Dr. Wagner shows that one year after hospitalization with TBI, women have more disability. Yet several animal studies utilizing experimental TBI and stroke models show that female hormones are neuroprotective in attenuating aspects of secondary injury such as excitotoxicity, ischemia, and oxidative stress. The primary goal of this project was to characterize, in a clinical population, possible gender differences in the production of CSF markers of secondary TBI and gender specific responses to hypothermia in attenuating these markers of TBI. Multivariate regression modeling techniques were used to show that females appear to have some neuroprotection against excitotoxic and ischemic injury. However, hypothermia appeared to reduce excitotoxic injury primarily in males. Ischemic injury and excitotoxicity markers were also linked to a marker of oxidative stress. Again there were significant gender differences in the relationship of ischemia/oxidative stress and excitotoxicity/oxidative stress. Females have much lower oxidative stress loads than males for a given excitotoxic or ischemic insult. These findings indicate that there may be acute clinical correlates to the early hormonally mediated neuroprotection previously reported in studies on experimental brain trauma. Portions of this work have been presented at the 2002 National and International Neurotrauma Society meeting. This work will be presented at the 2003 Association of Academic Physiatrists meeting. Manuscripts are currently in preparation and review. A large grant has been submitted as a part of the University of Pittsburgh's Center for Injury Research and Control's competitive renewal to the CDC based on this work. Collaborators include the NIH funded Brain Trauma Research Center CSF Bank (Ava Puccio MSN), Drs. Tony Fabio (Center for Injury Research and Control), Ross Zafonte and Hülya Bayır.

2. EE Promotes Cognitive Recovery in Male but not Female Rats after Experimental TBI

EE has been shown in a variety of animal models to improve behavioral performance and impact neural substrates affecting plasticity such as angiogenesis, neurotrophin production, gliogenesis, and dendritic sprouting. Enrichment of the housing environment has also been shown to improve spatial memory after experimental TBI in male rat models. However, the impact of gender on how EE affects behavioral performance after experimental TBI has not been studied. In this study, we used the CCI model of experimental TBI to determine the effects of EE on motor and cognitive behavioral performance for both male and female rats. The results of the study showed that early intervention with enrichment did not impair motor performance early after injury for either males or females. EE improved spatial memory performance for males only. This finding may be due to potential gender differences in enrichment-mediated neuroplasticity after injury or gender specific alterations in hormonal modulation of post-injury neuroplastic responses. The results of this work were presented by our undergraduate student, Joshua Sokoloski, in the 2002 National and International Neurotrauma Society student poster competition and recently published in *Neuroscience Letters*. This was the first enrichment study completed at the Safar Center, and it has served as valuable pilot data and treatment protocol for other investigators at the center

interested in pursuing other grant funding involving enrichment based studies. We are currently characterizing how enrichment and gender impact dopaminergic markers post-TBI. Future work will focus on other neural and hormonal substrates that may impact this gender specific behavioral response. Collaborators include Drs. E. Edward Dixon, Anthony Kline, and Ross Zafonte.

3. DA Kinetics and TBI

Altered DA neurotransmission is hypothesized to play a role in neurobehavioral deficits after TBI. DA agonists have been shown clinically to improve aspects of mental functioning after TBI, and have been shown in multiple animal studies originating from Dr. Dixon's laboratory to improve behavioral performance. This laboratory has also demonstrated reductions in striatal DA transporter (DAT) protein and increases in TH chronically after TBI. These proteins play a critical role in DA release and reuptake. However, the effects of DAT reduction and TH increases on DA neurotransmission are unknown. Fast scan cyclic voltammetry (FSCV) permits real time *in vivo* evaluation of DAergic kinetics. The goal of this project was to assess differences in striatal DA kinetics after experimental TBI using FSCV. In this study, we used the CCI model of experimental TBI to evaluate electrically evoked DA release as well as DA clearance 2 weeks after injury. Striatal dopamine release during bilateral electrical stimulation of the medial forebrain bundle was monitored in anesthetized rats by FSCV in conjunction with Nafion-coated carbon fiber microelectrodes. Striatal evoked release of DA was significantly lower on the injured side of the brain compared to the uninjured side two weeks after injury. First order, but not zero order, DA clearance kinetic profiles appear to be altered in the injured striatum. These findings provide a functional correlate to previously reported DA system protein changes. The relationship between behavioral performance, DAT concentrations, and DA kinetics will be studied. This work is being conducted in conjunction with Dr. Adrian Michael in the Department of Chemistry, whose research focuses on electrochemical techniques and the measurement of neurotransmitters using microsensor technology. A manuscript for this work is currently in preparation. We intend to investigate regional and post-injury time course differences in DA kinetics as well as response to acute and chronic pharmacotherapies. Collaborators include Drs. C. Edward Dixon, Adrian Michael, and Ross Zafonte.

4. The Impact of Gender and Hormonal Status after Experimental TBI

Some studies have shown that sex hormones have neuroprotective qualities in the setting of acute TBI. However, less is known about how endogenously circulating sex hormones or particular hormone levels at the time of injury effect behavioral performance. Here we used the CCI model of experimental TBI to evaluate how gender, pre-injury estrous cycle status, and pre-injury serum hormone status affect motor and cognitive behavioral performance after TBI. The results of this study showed that, for females, serum hormone levels and estrous cycle stage at the time of injury do not impact behavioral performance on any of the behavioral tasks. Females do significantly better than males on motor function tasks assessed early after injury. However, there were no significant

gender differences in spatial memory performance later after injury, implying that female sex hormones confer early neuroprotection with behavioral performance, but endogenous hormones may not significantly impact later behavioral outcome. A manuscript for this work is currently in preparation. Portions of this work will be presented at the 2003 Association of Academic Physiatrists. Future work will focus on how hormone manipulations affect behavioral performance and histochemical markers of injury. Collaborators include Drs. C. Edward Dixon, Anthony Kline and Ross Zafonte.

Gender Differences in Behavioral Performance with DA Enhancing Therapies

Several studies involving experimental TBI in male rats have shown that DA agonists improve cognitive recovery. Other literature shows that there are gender differences in how DA systems are modulated, and this modulation is largely affected by sex hormones. For example, striatal dopamine transporter concentrations are higher in females, and females are more behaviorally sensitive than males to dopamine agonists acting at the transporter. For this study, we used the CCI model of experimental TBI to evaluate the effectiveness of chronic treatment with the DA agonist, methylphenidate, on improving behavioral performance after TBI. The results show that daily treatment with 5mg/kg of Methylphenidate improves beam balance performance for both males and females. However, the degree of improvement with this task was much larger in the males, compared to the females. Additionally, Methylphenidate improved spatial memory for males but not females. The difference in cognitive performance with methylphenidate may, in part, result from an increased sensitivity to side effects in females compared to males to the drug at this dose. A manuscript for this work is currently in preparation. Future work will focus on how hormone and drug dosing manipulations affect behavioral performance. Collaborators for this study include Drs. Dixon, Anthony Kline, and Ross Zafonte.

5. Associations between DAT Genotype, Outcome, and CSF DA Levels after Severe TBI: A Preliminary Analysis

DA pathways have been implicated in cognitive deficits after TBI. While not associated with alterations in protein structure, the DAT genotypes are associated with differences in DAT protein density and development of DA mediated pathophysiological conditions such as attention deficit disorder. Differential DAT expression presumably affects both presynaptic DA release, via reverse transport, and DA reuptake. DAT regulation may have a role in DA mediated neurotoxicity acutely after TBI and play a compensatory role in improving DA neurotransmission chronically after TBI. Catacholamines, including DA and its metabolites, are subject to auto-oxidation, resulting in the formation of reactive oxygen species that can contribute to oxidative stress associated with secondary brain injury. Previous work from this laboratory has demonstrated reductions in DAT protein after experimental TBI. The role of DAT genotype on injury and outcome has not been studied. For this study, we evaluated 30 patients with severe TBI. We determined their DAT genotype, measured post-injury CSF DA and DA metabolite levels, and evaluated six-month neuropsychological outcomes. The DAT 10/10 genotype was considered the risk genotype for the production of more DA and DA metabolites as well

as for poorer outcome. Results showed no differences between genotype groups for post-injury CSF DA levels. However, there were significant increases in DA metabolite production for the DAT 10/10 genotype group compared to other genotypes. Results also showed that people with the DAT 10/10 genotype had worse six month disability scores and poorer performance with some neuropsychological tests involved with frontal lobe function. Future work will focus on repeating this analysis in a larger population. Additional work will focus on gender differences in DA CSF marker production and the relationship to DA CSF markers to outcome. This work was completed in collaboration with the University of Pittsburgh BTRC as well as Drs. C. Edward Dixon, Yvette Conley, Robert Ferrell, Sue Beers, Ross Zafonte, and Mary Kerr.

Support: NIH K08HD40833, Amy K. Wagner, M.D. PI, Dopamine Function and the Effects of Therapeutic Intervention $622,258 beginning 2001 for 5 years (Sponsors: C. Edward Dixon, Ph.D., Adrian C. Michael Ph.D, and Ross D. Zafonte, D.O.); NIH R03HD41399, Amy K. Wagner, PI Gender Differences in Dopamine Function after TBI $145,535 beginning 2002 for 2 years; CDC CCR310285-07---CIRCL Small Grants Program Amy K. Wagner, M.D. PI (Hank Weiss Ph.D. PI Center Grant) $7,000 beginning 2001 for Gender Based Associations with Outcome after Severe TBI, $10,000 beginning 2002 for Characterization of Alterations in the Female Rat Estrous Cycle after Experimental TBI; NIH P50NS30318 Clinical Core--University of Pittsburgh BTRC, C. Edward Dixon PI; Laerdal Foundation, Hülya Bayır PI, Oxidative Stress After Severe Head Injury; Department PMR, University of Pittsburgh.

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CARDIOPULMONARY ARREST PROGRAM

Research in cardiopulmonary arrest and resuscitation is carried out under the direction of Dr. Clifton Callaway at the University of Pittsburgh Center for Emergency Medicine. In addition, there are pediatric components to the cardiac arrest program that are being carried out by Dr. Robert Hickey of the Children's Hospital of Pittsburgh Division of Emergency Medicine and Dr. Howard Ferimer of the Mercy Hospital Department of Pediatrics.

A. Clifton Callaway and the Department of Emergency Medicine

Resuscitation from sudden cardiac arrest is dominated by two organs: heart and brain. Current clinical approaches for restoring the heart to a normal perfusing state are suboptimal. Only about one-third of resuscitations attempted by paramedics outside of the hospital result in restoration of circulation. This low success rate is in part because of inability to titrate drug and electrical therapy to the changing physiological sate of the patient. Subsequent to restoration of circulation, brain injury contributes to the demise of many more patients. Brain injury involves many biochemical changes that develop over the subacute period after reperfusion, and can be modified by therapy delivered during the first few hours after return of circulation.

Our studies employ both large and small animal models to study these two different components of resuscitation from normovolemic, sudden circulatory arrest. Using a swine model of cardiac arrest and resuscitation developed in the Department of Emergency Medicine by Dr. James Menegazzi, we are studying the optimal timing and delivery of drug and electrical therapy for restoring spontaneous circulation. Using a rat model of asphyxial cardiac arrest developed at the Safar Center, we are studying the biochemical changes in brain that develop over the hours following resuscitation.

Finally, our department has a long relationship and commitment to emergency medical services (EMS). Because EMS is the initial point of care for most patients with sudden cardiac arrest, it is an essential clinical arena for any study of this disease. We continue to cultivate this resource for future translational research.

1. Altered Intracellular Signaling in Brain after Resuscitation

Intracellular signaling in rat brain after cardiac arrest has been studied courtesy of an NINDS Independent Scientist Award. We previously described changes in intracellular signaling in rat brain after resuscitation from eight minutes of normothermic asphyxia, resulting in five minutes of circulatory arrest. In particular, the activity of two mitogen-activated protein kinases (MAPKs) increase in hippocampus over the 24 hrs period after reperfusion: the p42/p44 MAPK (extracellular-signal regulated kinase, ERK) and the Jun-N-terminal kinase (JNK). Induction of mild hypothermia (33°C) between 1 and 23 hrs after reperfusion, selectively increases activity of ERK relative to normothermic (37°C) controls. This regimen of hypothermia also decreases histological and behavioral signs of

brain damage, prompting us to speculate that some of the beneficial effects of induced hypothermia are mediated via increased ERK activation.

Immunohistochemistry and immunoblotting reveal a similar pattern of MAPK activation after cardiac arrest and reperfusion in other brain regions. Interestingly, the influence of hypothermia on ERK activation during the first 24 hrs of reperfusion appears to be more pronounced in forebrain (cortex and hippocampus) than in hindbrain (cerebellum and brainstem). Because the forebrain structures are more sensitive to ischemic injury, this regional selectivity further supports a relationship between ERK activation and the neuroprotective effects of hypothermia.

Further studies sought to identify the upstream activators of ERK, as well as the downstream effectors of the biological activities of ERK. Potential upstream activators of ERK include several neurotrophic factors. In the hippocampus, immunoblotting revealed no change in levels of nerve growth factor or neurotrophin-3. However, brain derived neurotrophic factor (BDNF) appears to increase after ischemia, and this increase is potentiated by induced hypothermia. These findings were reported in the *Journal of Cerebral Blood Flow and Metabolism.*

The biological effects of ERK are mediated by activation of a variety of transcription factors that can be measured using immunoblotting. Induction of hypothermia increases activation of factors believed to be substrates of the extracellular signal regulated kinase (ERK), including ATF-2 and CREB. Activation of CREB may be related to an increase in activation of the p90 ribosomal S6 kinase, a kinase that is itself a substrate of ERK.

In order to determine whether these changes in transcription factor activation are reflected by altered gene transcription, we have spent the last few months developing tools for assessing global patterns of mRNA expression. In our first experiments, hippocampi were collected 24 hr after asphyxial cardiac arrest and reperfusion at 37°C (n=3). Sham rats (n=5) underwent anesthesia and operation without asphyxia. Total hippocampal RNA from each group of rats was reverse transcribed into radiolabeled cDNA. This cDNA probe derived from total hippocampal RNA was hybridized with a nylon membrane spotted with 1176 oligonucleotides complementary to known rat genes. Autoradiograms of this membrane revealed specific changes in expression of a subset of genes. Using a criterion of 50% change in expression on more than one array, we detected 15 genes with increased expression after ischemia/reperfusion. These genes included heat shock proteins, tyrosine kinase related proto-oncogenes, and genes related to lipid metabolism. Expression of 53 genes decreased after ischemia/reperfusion. These genes included many products related to neurotransmitter synthesis, neurotransmitter receptors, and intracellular receptor signaling. These data indicate that circulatory arrest and reperfusion triggers a specific pattern of gene expression in rat hippocampus.

Other experiments have been directed at developing a satisfactory pharmacological tool for inhibition of ERK activation during reperfusion after ischemia. The compound

SL327 worked well after intravenous administration, but remains unavailable from the manufacturer. Geldanamycin, an inhibitor of the MAPK kinase kinase, Raf, also did not penetrate the blood-brain barrier and proved to be toxic after intraventricular doses large enough to penetrate into the brain parenchyma. We conducted an intravenous dose-response study of the MAPK kinase inhibitor U0126 (0.2 – 2.0 mg/kg), which has been used by others in rodent studies of ischemia, and determined that this compound did not decrease ERK activation in the brain. It is likely that this compound does not cross the blood-brain barrier.

Our future directions will be to test for a causal link between increased ERK activation and the improved neurological outcome observed after induced resuscitative hypothermia. This test will require us to establish a reliable pharmacological tool for inhibiting ERK activation. Likewise, we will test whether the increased levels of BDNF are necessary and sufficient for the beneficial effects of hypothermia, by stimulating and antagonizing this pathway.

2. Use of ECG Waveform Analysis to Guide Resuscitation From Ventricular Fibrillation

The optimal timing of rescue shocks for reversal of ventricular fibrillation (VF) requires clarification. Certainly, rapid defibrillation is optimal for restoring circulation after brief periods of circulatory arrest. However, a period of reperfusion prior to rescue shocks may be a superior approach for prolonged periods of circulatory arrest. We have previously developed a metric for quantifying organization in the ECG during VF that is called the scaling exponent. The scaling exponent can discriminate between animals with brief periods of circulatory arrest (in which rescue shocks have a high likelihood of success) and animals with prolonged periods of circulatory arrest (in which rescue shocks have a low likelihood of success).

With support from Physio-Control Medtronic, Inc. to Dr. James Menegazzi, we have examined whether the scaling exponent calculated in real-time could be used to differentiate swine with VF that benefit from rescue-shock-first from those that benefit from reperfusion-first. Preliminary results of this study reveal that when the scaling exponent is low (after 1-4 minutes of circulatory arrest), beginning resuscitation with rescue shocks is effective for restoring circulation. In contrast, when the scaling exponent is higher (after 8-12 minutes of circulatory arrest), beginning resuscitation with countershocks only restored circulation for 50% of swine. The alternative strategy of beginning resuscitation with chest compressions and pressors for up to 5 minutes before the initial rescue shock restored circulation for 81% of swine. Moreover, spontaneous circulation was restored more quickly in these latter swine, despite the built-in delay prior to the first rescue shock. These data suggest that a real-time measurement of the VF waveform could be used to guide the timing of the initial rescue shock to be delivered during resuscitation from a VF cardiac arrest.

In order to determine whether this type of VF analysis will be useful during human cardiac arrest, we have also analyzed recordings of VF cardiac arrest obtained from automated external defibrillators. We previously reported on an analysis of similar recordings from Pittsburgh. This year, we obtained a larger sample of recordings from a separate EMS system. Preliminary analyses of these data confirm that the scaling exponent can predict the likelihood of successful rescue shocks. When the VF waveform preceding a rescue shock has a low scaling exponent, the rescue shock is likely to result in defibrillation. However, when the scaling exponent is high, the rescue shock is likely to fail. Because repeated failed rescue shocks may be detrimental to cardiac function, this measure could provide a real-time monitor for rational application of electrical therapy during resuscitation. Anecdotal observations in these recording confirm that artificial reperfusion with chest compressions and pressors can restore VF with a high scaling exponent to VF with a low scaling exponent.

3. Altered Blood Coagulation after Circulatory Arrest.

Previous studies have shown that fibrinogenesis is elevated during and after cardiac arrest. Furthermore, this increase is not matched by a concomitant increase in fibrinolysis. It is not currently known if increases in fibrinogenesis occur during the period of no-flow, during low-flow during resuscitation, or during subsequent reperfusion. Dr. Hostler, an EMS fellow, examined thrombin-antithrombin III (TAT) levels, a marker of fibrinogenesis, during resuscitation and reperfusion in the swine model of cardiac arrest. There was a time-dependent increase in TAT levels over ten minutes of untreated cardiac arrest, and the increases were most pronounced after six minutes of cardiac arrest. TAT levels were elevated at the beginning of resuscitation (132% of baseline) and increased further at ROSC (523%) (5.2 ± 1.0 min later). This elevation persisted after 30 and 60 minutes of reperfusion. Thus, TAT levels rise in proportion to the duration of circulatory arrest beyond six minutes, and remain elevated during resuscitation and after reperfusion. These data were presented at the Society for Academic Emergency Medicine in St. Louis, MO.

Because the increases are persistent, even when sampled after reperfusion, TAT levels or other markers of thrombogenesis could be used to estimate the combined duration of no-flow and low-flow prior to resuscitation. In future clinical studies, this type of marker would be useful for stratifying samples into groups with brief versus prolonged cardiac arrest. Dr. Hostler has initiated protocols to confirm these observations in human subjects being treated by our local EMS service. Further work is required to establish the cause and pathophysiological significance of these changes.

4. Clinical Studies

The various issues surrounding research on emergency care of human subjects who are unable to provide consent have been well described. Criteria for waiving the requirement to obtain informed consent in emergency research have been set forth. In order to lay the groundwork for translation of our basic science investigations to patients, Dr. Margaret

Hsieh studied 100 consecutive responses by City of Pittsburgh paramedics in order to better establish a case for one of these criteria in our target population. In this study, we determined that individuals who could serve as surrogate decision-makers for subjects being treated for cardiac arrest were only available in about one-half of cases. Furthermore, these surrogate decision-makers could only be located after about 25 minutes of resuscitation, and were usually unable to answer simple questions because of their emotional state. Thus, we have established that the mechanism of waiver of informed consent, rather than reliance on a surrogate or proxy is appropriate for most research in out-of-hospital cardiac arrest, and absolutely required for studies of therapy with a therapeutic window of less than one-half hour after beginning resuscitation. These data were presented as a Brief Communication in *Academic Emergency Medicine*.

Dr. David Newman, an EMS fellow in our department, conducted a further study to test our capacity for collecting detailed data during resuscitation from out-of-hospital cardiac arrest. In this study, a cerebral oximeter (provided by Somanetics, Inc.) was placed on 16 patients as soon as possible during resuscitation attempts. This device provides a measure of arterial and venous blood oxygen saturation in the brain, and this measure correlates with jugular venous oxygen saturation. During chest compressions, the cerebral oximeter readings were below the lower limit of detection, suggesting that there is little if any oxygen delivery during typical resuscitations. Interestingly, the cerebral oximeter was a very sensitive detector of the return of spontaneous circulation, often reporting increasing values prior to detection of pulses by the treating team. After reperfusion, oximetry readings increased and decreased with blood pressure, suggesting that cerebral perfusion after resuscitation lacks autoregulation, and is supply-dependent. This pilot study suggests a potential role for cerebral oximetry in titration of hemodynamics after reperfusion.

5. Plans

Research efforts will continue to focus on the molecular aspects of brain injury after cardiac arrest and on the immediate clinical questions of acute cardiac resuscitation during cardiac arrest. The rodent research to this point has led us to the question of what role do neurotrophic factors, signaling kinases, and new gene expression play in the response to cardiac arrest? Furthermore, what is the specific interaction between these events and the beneficial effects of post-reperfusion hypothermia? Swine studies will continue to refine the optimum approach to restoring circulation after prolonged circulatory arrest. We hope to recruit additional clinicians interested in translation of our basic knowledge about the situation of sudden cardiac arrest to the practical care of patients.

B. Pediatric Cardiopulmonary Resuscitation

1. Public Education and National Guidelines Committee

Dr. Robert Hickey is the current chair of the American Heart Association subcommittee on Pediatric Resuscitation. The subcommittee is responsible for overseeing the American Heart Association's pediatric advanced life support (PALS) course. PALS is taken by approximately 150,000 healthcare providers per year. The latest revision to the course was distributed in December 2001 with 153,000 PALS textbooks sold in the first 8 months of release. In his capacity as chair of the Pediatric Subcommittee, Dr. Hickey also serves as a representative to the international liaison committee on resuscitation (ILCOR) and has recently participated in meetings in Melbourne, Australia and Florence, Italy to develop international consensus on new developments in resuscitation science.

2. Laboratory Research in Pediatric Resuscitation

Drs. Robert Hickey and Howard Ferimer are principal investigators on the rodent asphyxial arrest projects.

A. Developmental aspects of COX-2-mediated brain injury

Dr. Robert Hickey continued work on his KO-8 award from NICHD to study developmental aspects of the role of COX-2 in brain injury. This research is being carried out under the mentorship of Steven Graham in the department of Neurology at the VA Hospital. Dr. Kochanek is a co-sponsor of the grant. COX-2 plays an important role in secondary injury in models of stroke, trauma, and cardiac arrest in adult investigation. Its role in pediatric brain injury remains to be defined.

Support: COX-2 and Injury in the Immature Brain, KO-8 (#HD40848) National Institute of Health, National Institute of Child Health and Development, (7/01-7/06), total award $623,430 ($115,450 direct + $9,236 indirect per year), Robert W. Hickey, M.D., PI, Steven Graham, M.D., Patrick Kochanek, M.D., Co-Investigators; Robert Clark, M.D., C. Edward Dixon, Ph.D., Peter Safar, M.D., Consultants.

B. Role of Adenosine after Asphyxial Cardiac Arrest

Adenosine is produced by the breakdown of ATP during ischemia. It is neuroprotective through multiple mechanisms; reduction in free radical production, hypothermia, improved cerebral blood flow and reduction in cellular metabolism. Systemic administration of adenosine is limited by its short half-life, inability to cross the blood brain barrier and the adverse cardiovascular side effects of hypotension and bradycardia. The beneficial neurologic effects of augmenting adenosine levels locally in the brain have been documented in TBI and Stroke models of cerebral ischemia. Whether augmenting adenosine levels in the brain after asphyxial cardiac arrest is beneficial is not known. It is the focus of these investigations.

In collaboration with Dr. Edwin Jackson, Dr. Ferimer is continuing to study the effects of adenosine modulating drugs on interstitial levels of purines in the brain of animals

subjected to asphyxia-induced cardiac arrest. We employed state-of-the-art microdialysis and analytical methods, including high-performance liquid chromatography, to conduct these detailed studies. Our investigations are addressing pharmacological strategies to increase brain adenosine levels in the acute phase after resuscitation.

Our future work will focus on employing these methods to improve neurological and histological outcome following resuscitation from asphyxia-induced cardiac arrest. Ultimately, our studies may improve therapy in this all-to-common problem in the pediatric population.

3. Pediatric Cardiopulmonary Arrest: Clinical Studies

Dr. Hickey has initiated the assembly of a multidisciplinary team to evaluate children resuscitated from cardiac arrest. The team has representatives from the entire continuum of care including pre-hospital, emergency medicine, critical care, neurology, neuroimaging, behavioral pediatrics, and rehabilitation medicine. The team will, 1) characterize early molecular markers of HI brain injury, 2) evaluate strategies for prognosis of neurologic recovery, 3) identify patterns of functional deficits in long-term survivors, and 4) develop targeted strategies for rehabilitation of patients with HI brain injuries. This information will facilitate comprehensive evaluation and treatment for individuals suffering from HI brain injury and also develop a profile of the natural history of injury and recovery that can be used for evaluation of anticipated neuroprotective therapies.

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SHOCK AND SUSPENDED ANIMATION PROGRAM

Drs. Peter Safar and Samuel Tisherman

"Novel resuscitation from lethal hemorrhage; increasing survival of combat casualties" is a Department of Defense (DOD) supported program which began in 1997 and was, during 2001/2002, in its fourth year. It consists of project I on hemorrhage shock (HS) in rats and pigs (P.I., Dr. Tisherman; Co-P.I., Dr. Safar); and project II on suspended animation (SA) in dogs (P.I., Dr. Safar; Co-P.I., Dr. Tisherman). For the overall program in 1997-2002, Dr. Safar has been P.I. and Dr. Tisherman Co-P.I. The funding since 1997 was made possible through special "plus-up" funds from congress initiated by former Navy Commander Lyn Yaffe, M.D. His successor during years 3-4 was Jeannine Majde-Cottrell, Ph.D. Each year requires a new proposal, which is peer-reviewed by a DOD committee. For the two programs combined, we received total funds (including 50% institutional "indirect costs") of approximately $2.4M for year 1; $1.9M for year 2; $1.2M for year 3; and $1.1M for the current year 4. The most expensive part of the budget are team and animals for the research intensive care unit (ICU) studies in dogs.

Our research ICU for large animals, initiated in the 1970s, is still considered a unique resource for the documentation of novel CPCR methods. It must be maintained continuously to be cost-effective, with at least four technicians, two full-time MD research fellows with CCM experience, and about 80 long-term dog experiments per year. Maintaining this ICU program alone requires over $0.5M per year. In 2001/2002, the research fellows were Dr. Nozari (in his first year) and Dr. Wu (in his third year); Mr. William Stezoski has continued as lab coordinator. The co-investigators or consultants included Drs. Kochanek, Klain, Jackson, Dixon, Clark, Kagan, Jenkins, and Radovsky (pathologist). Outside consultants included Dr. Hsia of the Synzyme Corporation, Dr. Taylor of the Organ Recovery Systems, Inc., Mr. Samson of the Cardeon Corp. (cardiopulmonary bypass catheters and other devices), and others. In spring 2001, Dr. Safar divided the projects' funding by having the HS projects continue with Dr. Tisherman as P.I. and Dr. Safar as Co-P.I., funded by an intramural grant from the Navy (ONR). The congressional plus-up money continued supporting the SA project, with Dr. Safar as P.I. and Dr. Tisherman as Co-P.I.

The objective of the HS-SA program has been to help maximize the reversibility of presently lethal traumatic hemorrhage. The HS studies in rats and pigs were to extend the golden hour of HS tolerance; HS (low blood flow), with viscera as the most vulnerable organs, is the prevalent cause of death in soldiers "dying of wounds" (DOW). Exsanguination cardiac arrest (CA) (no blood flow), with the brain as the most vulnerable organ, is the prevalent cause of death in soldiers "killed in action" (KIA). SA is a totally new approach for presently unresuscitable conditions. While SA has been considered science fiction, colleagues are now increasingly using this term seriously, as representing rapidly induced preservation of the organism for delayed resuscitation. This idea was initiated in the 1980s by Drs. Bellamy and Safar. For HS and SA we explored pharmacologic as well as hypothermic strategies – specifically mild hypothermia (33-36° C) for HS and profound hypothermia (5-15° C) for CA. Dr. Tisherman is planning,

for both, a clinical feasibility study in selected trauma hospitals to start in the near future. Devices will have to be developed by outside companies under the guidance of our team.

The HS models in rats and SA models in dogs used in 2002/2003 had been initiated and further developed over the years by our group. They have several unique features, the most important being clinical relevance in terms of outcome.

1. Hemorrhagic Shock (HS) Studies (Tisherman)

The hemorrhagic shock studies of academic year 2001-2002 were completed under year 5 of funding by the Office of Naval Research (PI: Samuel A. Tisherman, MD; Co-PI: Peter Safar, MD). A total of 267 rats were used. The studies were completed by technician Jason Stezoski under direct supervision of fellow Xianren Wu, MD.

Spontaneous Cooling during HS

In previous studies of hypothermia during HS in animal models, normothermia was maintained until therapeutic hypothermia was induced. In trauma victims, however, spontaneous hypothermia is common. The clinical issue for resuscitative hypothermia may be more a question of whether or not to *rewarm*, than to actively *cool* since the patients will already be mildly hypothermic. We hypothesized that after spontaneous cooling during HS, continuing mild, therapeutic hypothermia during resuscitation is beneficial. We felt that this study was important before considering clinical trials of hypothermia. After moderately severe HS (pressure-controlled HS at mean arterial pressure (MAP) 40 mm Hg until 30% reuptake of blood is needed), only 3 of 8 rats in the normothermia group survived to 72 hr, whereas 2 of 8 hypothermic rats survived. Most deaths were after 24 hr. After more severe HS (to 50% reuptake), survival time was longer with continued hypothermia. It appears that mild hypothermia is more beneficial to prevent early deaths, presumably from cardiopulmonary dysfunction, than late deaths, presumably from the systemic inflammatory response and multiple organ failure. This study was presented as a poster at the American Association for the Surgery of Trauma meeting in Orlando, FL. The manuscript is in preparation for the *Journal of Trauma*.

Very prolonged HS

Thus far in year 5, we have focused on development of a model of very prolonged HS in rats. The goal is to develop a resuscitation strategy for trauma victims that require prolonged extrication and transport times as in the military and rural settings. In the first part of this study, using a model of uncontrolled HS via tail cut for 6 hr, we compared target MAP of 50 vs 60 vs 70 mmHg for limited fluid resuscitation with whole blood (shed plus donor). Mortality increased with lower MAP goal, but there was significant variability in MAP. To control MAP more precisely we are currently repeating the study using a more classic Wiggers-type pressure-controlled HS model including systemic heparinization. An abstract for this study has been submitted to the Society of Critical Care Medicine.

Dr. Wu presented 3 posters at the meeting of the Society of Critical Care Medicine, January 26-30, 2002, entitled "Mild hypothermia (34° C) does not increase initial bleeding from the injured liver after HS in pigs, Resuscitation with Ringer's ethyl pyruvate solution (REPS) fails to improve long term survival compared to lactated Ringer's (LR) solution after severe volume-controlled HS in rats," and "Inhibition of sodium/hydrogen ion (Na+/H+) exchange with methyl isobutyl amiloride impairs tolerance to hemorrhagic shock in rats." At the same meeting, Dr. Tisherman presented a poster entitled "Critical care experiences during surgical residencies do not affect surgical practice." Dr. Rainer Kentner presented a paper at the American Society of Anesthesiologists meeting entitled "Doubling the "golden hour" of traumatic HS tolerance with mild hypothermia and an anti-oxidant." Dr. Tisherman participated in a debate (with Larry Gentilello, MD) regarding the pros and cons of resuscitative hypothermia at the Advanced Technology Applications for Combat Casualty Care meeting in Fort Walton Beach, FL, on September 10, 2001. He also gave the following invited talks: "SA Research" at the Association of Neurological Surgeons, Chicago, IL, 4/11/02; "Hypothermia for Resuscitation from Trauma: It's Cool to be Cool" at the Society of Air Force Clinical Surgeons, Las Vegas, NV, 4/19/02, and at the Washington DC Area Critical Care Society, Washington, DC, 6/13/02. "Development of an Evaluation Instrument for Surgical Crisis Resource Management" at the Association for Surgical Education, Surgical Education Research Fellowship Forum, Baltimore, MD, 4/5/02.

2. Suspended Animation (SA) in Dogs (Safar)

In 2001/2002 we continued to search in dogs for breakthrough effects among 14 different drugs and different temperatures, added at the start of CA to the saline flush into the aorta. Alone, without drugs, when we delivered saline at 0-4° C, we achieved considerable preservation and resuscitation. The drugs were selected according to one or more of six mechanistic strategies, documented by us and others in the past, and on the basis of published results in rodents and advice from co-investigators and consultants. Almost all drugs tested had not yet been evaluated for outcome after prolonged CA in large animals. We were seeking a breakthrough effect, namely overall performance category (OPC) = 1 at 72 hr, i.e., functionally normal dogs, in the majority of experiments of exploratory mini-series. OPC = 1 was achieved in very few experiments. With exsanguination CA 20 min no flow, only mild hypothermic aortic arch flush with the antioxidant *Tempol* resulted in OPC 1 or 2 (good outcome) in all 8 dogs. Tempol flush was also effective in other settings. Strangely, Tempol improved function but not histologic brain damage. An explanation for this is speculative. Some mechanism studies were added. Treatment with any of 11 other drugs resulted only in dogs achieving OPC 3 or 4, severe neurologic deficit and severe histologic damage. Thus, using 84 exploratory dog outcome experiments with the same model by the same team, only the water soluble antioxidant Tempol (which crosses the blood brain barrier) improved neurologic outcome.

We then used the same models for exploring *hypothermic strategies,* without drugs, merely using flush of isotonic saline, pushing the insult beyond that of the previous years. We extended the CA no flow period from 20 min and 30 min, to 60, 90, and 120 min. Some results were "firsts." Saline at 2-4° C, flushed with a 1 l/min flow rate into the aorta, decreased Tty by 3° C/min. This is faster than any other cooling method, except for using cardiopulmonary bypass (CPB). With CA 20 min no-flow, aortic arch flush rapidly lowered tempanic temperature (Tty) to 34° C and achieved survival to 72 hr with functional normality (OPC = 1) and histologically minimal damage. A delay in flush during normothermic CA to 8 min no-flow before start of cold-flush negated the preservation achieved with flush starting at CA 2 min or 5 min. When we increased CA to 30 min no flow we found that the flush volume of saline at 4° C had to be increased to 100 ml/kg to achieve functionally normal brains. This in some dogs achieved even histologically normal brains. The aortic catheter had to be withdrawn into the abdominal aorta to also protect by cold flush the viscera and spinal cord against ischemic damage. Aortic flush to Tty 20° C, 15° C, or 10° C preserved the brain and organism long enough to achieve intact survival (OPC 1) after 60 min, 90 min, and in some dogs even 120 min no flow. We now know that with Tty 10° C (induced with saline 0-4° C flush starting at CA 2 min) one can count on full preservation of all organs' viability after up to 90 min no flow. All 6 dogs with CA 90 min and Tty 10° C were functionally normal. One dog after CA 60 min, one after CA 60 min, one after CA 120 min, and one normal dog received cognitive function tests months later; these were normal. Functional and histologic studies of extracerebral organs after 72 hr have been initiated. CA up to 60 min was survived with intact viscera; liver function values were abnormal only transiently.

In the last study, with CA 120 min, when flush was with theoretically optimized solutions instead of saline – namely Normosol, Unisol, and Tempol *combined* -- all 6 dogs achieved good outcome. Thus there was a small additional outcome benefit in using solutions other than saline for the cold flush, stasis, and reperfusion. Polynitroxylated albumin with Tempol (Synzyme Co.) gave slightly better neurologic outcome. Unisol (Dr.Michael Taylor) resulted in easier restoration of stable spontaneous circulation. We found that the antioxidant Tempol is more effective when given at the beginning of HS or CA than when given during reperfusion.

We are working in communication with Drs. Peter Rhee and Hasan Alam of the Uniformed Services University of Health Sciences Office of Naval Research (USUHS-ONR), who were inspired by our work. They researched SA for over 3 hr with asanguinous low flow (not CA), using CPB via thoracotomy in pigs.

In spite of Dr. Safar's three life-threatening operations in winter 2001/2002, we visited in summer 2002 the U.S. Army Medical Research and Materiel Command/Telemedicine and Advanced Technology Research Center (USAMRMC/TATRC) in Washington, D.C. A team led by Dr. Safar presented the SA project's present and future plans and had funding for years 2003 reviewed and approved. In April 2002, Drs. Safar and Nozari presented cerebral topics at the American Society of Neurology meeting in Denver.

The SA dog projects in 2001/2002, using 3-4 day long outcome experiments, conducted 80 experiments. These experiments were under the team leadership of Dr. Nozari, supported by technicians Jeremy Henchir, Sherman Culver, Scott Kostelnik, Alan Abramson, and Murugan Subramanian: 1) Special aortic SA flush solutions (Unisol; Tempol) gave better outcome results than saline. 2) Exsanguination CA of 60 min no flow is more difficult to reverse to intact survival if the insult includes trauma; the latter causes coagulopathy. 3) SA with aortic flush using recirculated cooled, diluted venous blood gives better outcome than one-way cold saline flush. 4) We simulated "unresuscitable" hearts with prolonged CPB, in prolonged normovolemic ventricular fibrillation (VF)-CA, mild hypothermia during prolonged CPCR steps A-B-C, using veno-venous cooling, gave significantly better outcome data after mild hypothermia, in one study with 40 min steps airway (A)-breathing (B)- circulation (C) and another with 60 min steps A-B-C.

3. Miscellaneous

We have coached development of devices and methods for rapid vessel access and portable pumping-cooling. The latter is being initiated by the Biocontrol Company of Western PA, guided by us. Dr. Miroslav Klain received a spinoff grant in the fall of 2001 for helping develop a "smart catheter" and vessel access -- $0.1M from the Army via Illinois Institute of Technology Research Institute (IITRI). Additional funds for the catheter project are to Dr. Lynn Yaffe as PI; the Cardeon Co. to produce the catheter, and to IITRI to produce the sensors.

We guided devices developments needed to move SA into the out-of-hospital arena. Five years ago two patents had been obtained by Drs. Safar, Klain, and Mr. Stezoski for the University of Pittsburgh, one for a portable multi-modular cardiopulmonary bypass apparatus, and the other for single or double balloon aortic catheters, as licensed to the Cardeon Corp. of California. The company has used these patents so far for the development of a "Cobra Catheter" for heart surgery. They are now planning to go into CPB for resuscitation.

Drs. Safar and Klain and Mr. Stezoski have been urging the Biocontrol Co. of Western Pennsylvania to develop a portable device for rapid cooling and pumping of blood and fluids. In patients after CA and ROSC, with circulation, there is an urgent need for rapid lowering of brain temperature to mild hypothermic levels (from 38 to 34° C). Our team found in dogs with circulation, with 10% cardiac output pumped through an improvised cooler, to achieve mild hypothermia within 6 min. For patients with cardiac arrest (SA), we are coaching industry to develop a rapid delivery system for aortic cold flush.

Since 2001, we co-initiated and helped the NIH PULSE initiative to include in resuscitation research, traumatology. Our group led new initiatives at local, national, and international levels for boosting the weakest link in civilian EMS, life supporting first aid (LSFA) skill acquisition by the public. Among several efforts to advance resuscitation delivery, Dr. Safar moved LSFA training programs with self-training systems into

Pennsylvania and perhaps soon beyond, by guiding the Save a Life Foundation (SALF) of Chicago (Carol Spizzirri, R.N.) in its move to Pittsburgh. Dr. Steven Orebaugh, anesthesiologist, emergency physician, and educator, assumed the chair of a community LSFA committee. That program will be housed in the WISER, which, chaired by Dr. John Schaefer, is formally affiliated with the Safar Center.

Dr. Tisherman is promoting better understanding of controlled mild hypothermia vs. uncontrolled accidental cooling in patients with traumatic hemorrhagic shock. Drs. Safar and Behringer, and SCRR visiting professor Dr. Bernd Boettiger of Heidelberg, Germany, jointly represented cerebral resuscitation at the Wolf Creek conference for resuscitation research in June 2001 in Palm Springs, CA. The highlights of the HS-SA projects of 2001 will be presented in seven posters/talks at the forthcoming SCCM meeting in January 2002 in San Diego.

An important extension of the fruits of past CA research by Dr. Safar, Dr. Tisherman, and other team members, has been the following: Our group's discovery and documentation in dogs of mild resuscitative hypothermia after prolonged normothermic CA in dogs (1987-1994), has led to randomized clinical outcome studies abroad. This was not possible in the USA where the needed waiving of prospective informed consent was outlawed. Our alumnus Sterz of Vienna and colleagues in Australia and Japan have obtained statistically positive outcome data with mild hypothermia after prolonged cardiac arrest in patients. The two key papers have been published in the *New England Journal of Medicine* in early 2002. In 2001, the journal invited Dr. Safar to write an editorial on this subject which will appear (by Safar and Kochanek) in the same issue with the two clinical trials reports. This will create a boost for resuscitative hypothermia for various indications.

On September 11, 2001, Drs. Safar and Tisherman were returning from a DOD meeting in Fort Walton Beach when the Attack on America occurred. Dr. Safar delegated to Dr. Doris Cope his lecture for the Fifth International Symposium on the History of Anesthesia in Santiago de Compostela, Spain, on September 20, 2001. His lecture was on "Development of Cardiopulmonary-Cerebral Resuscitation in the Twentieth Century." Dr. Safar presented and discussed resuscitative hypothermia at the Cleveland Clinic's Neurocritical Care 2001 Conference on September 29, 2001. At the American Society of Anesthesiologists Congress in October 2001, Dr. Safar discussed suspended animation at a panel meeting with major interest from the media. In April 2002, Drs. Safar and Nozari presented "CPCR, From Animal Models to Clinical Trials" at the American Academy of Neurology.

Dr. Safar continued guiding the SA laboratory activities in spite of major operations in October 2001, November 2001, and May 2002. In March 2002, Dr. Safar's work was featured in the Pittsburgh Post-Gazette. In April 2002, Dr. Safar was visiting professor in Vienna to help plan the world's second large animal research ICU. Finally, in the summer of 2002, Dr. Safar prepared a major slide series on the SA project for the *DOD.*

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